

FIG. 1A

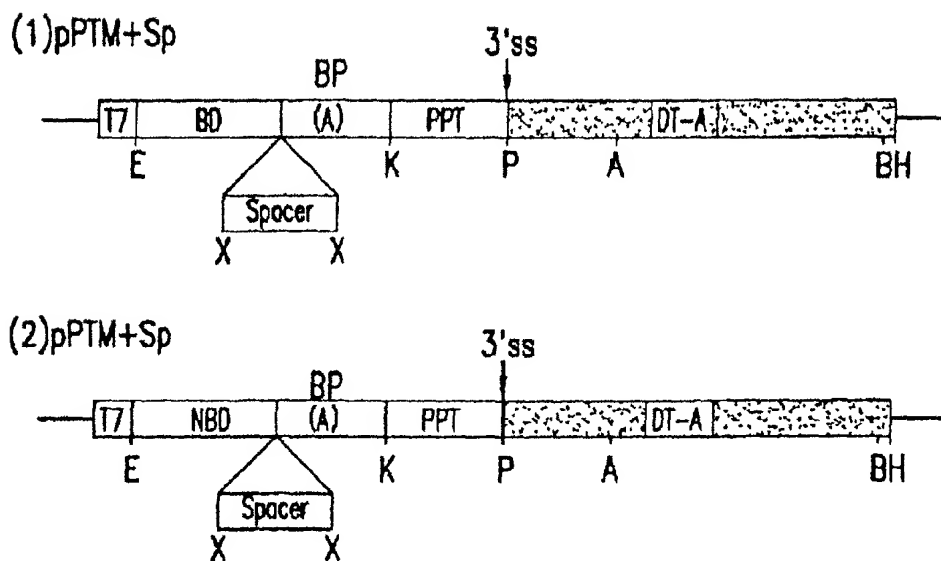


FIG.1B

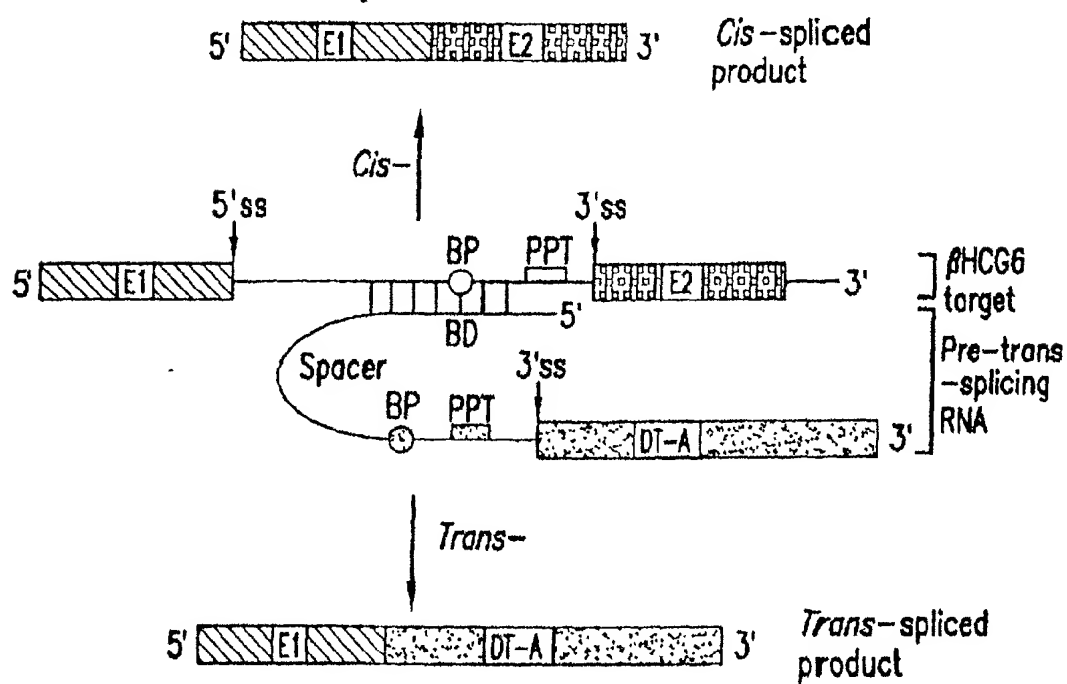


FIG.1C

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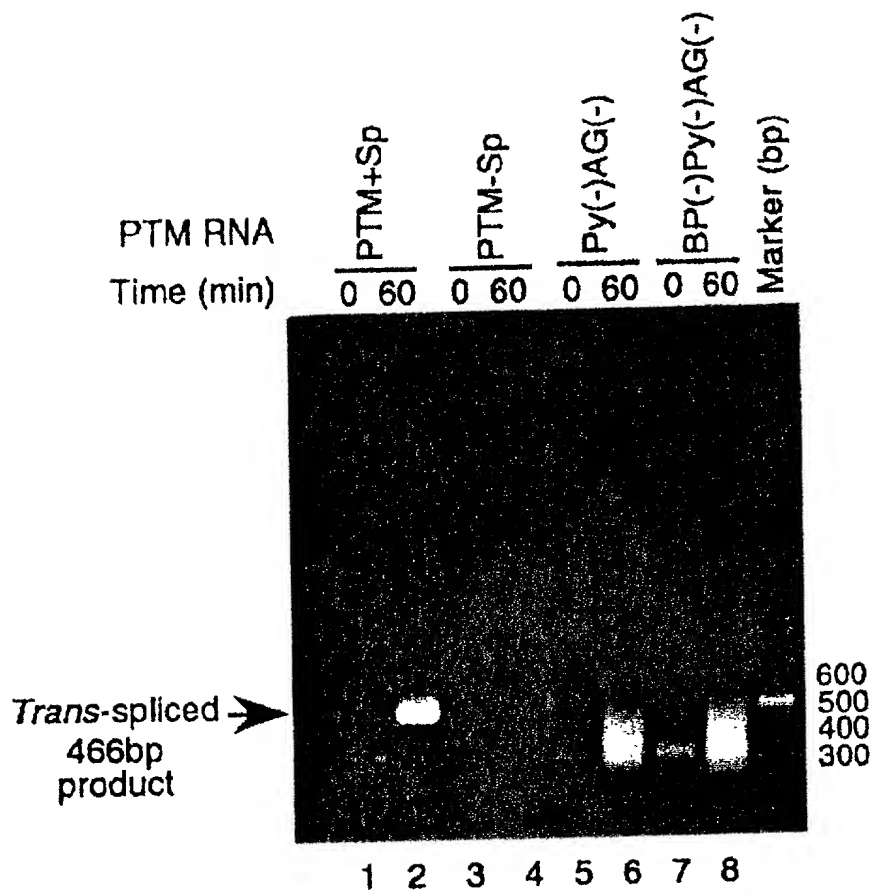


FIG.2A

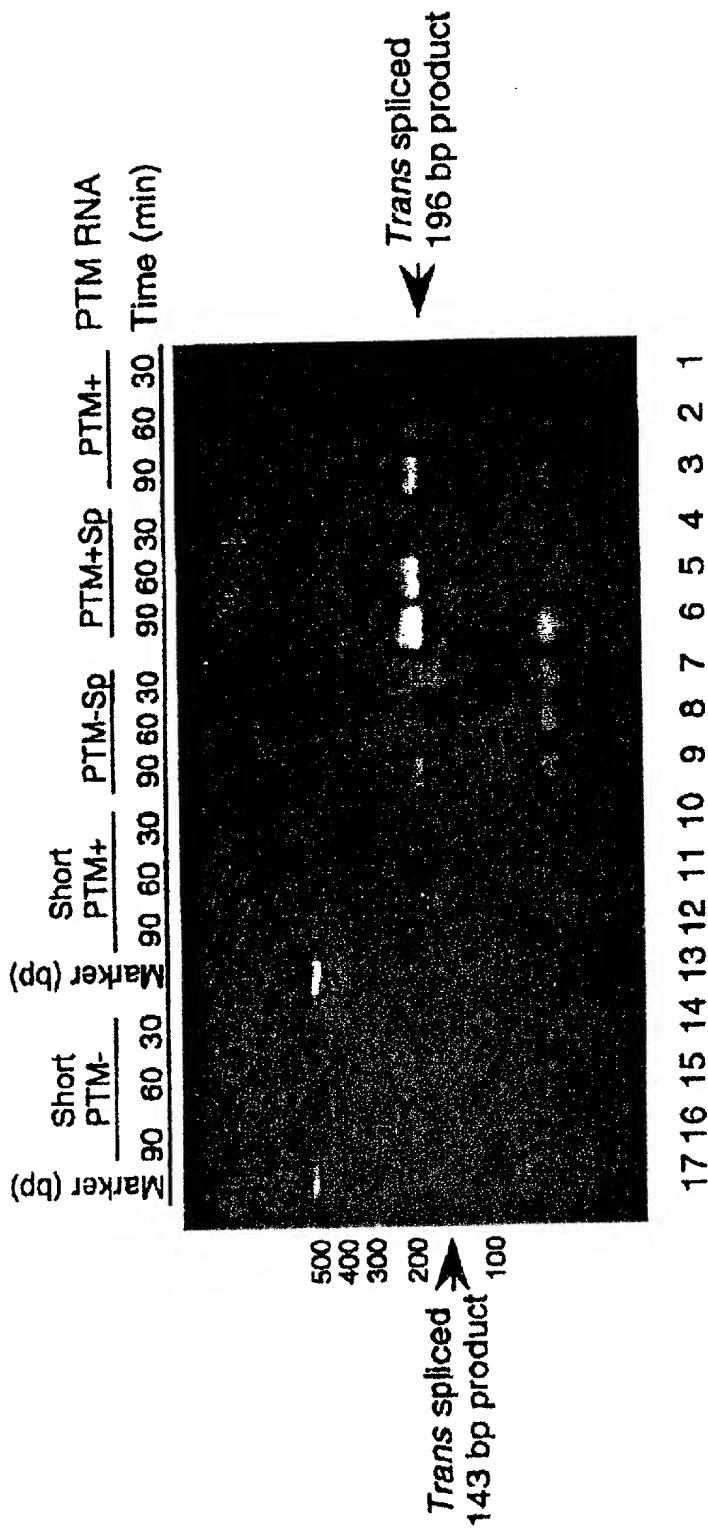


FIG.2B

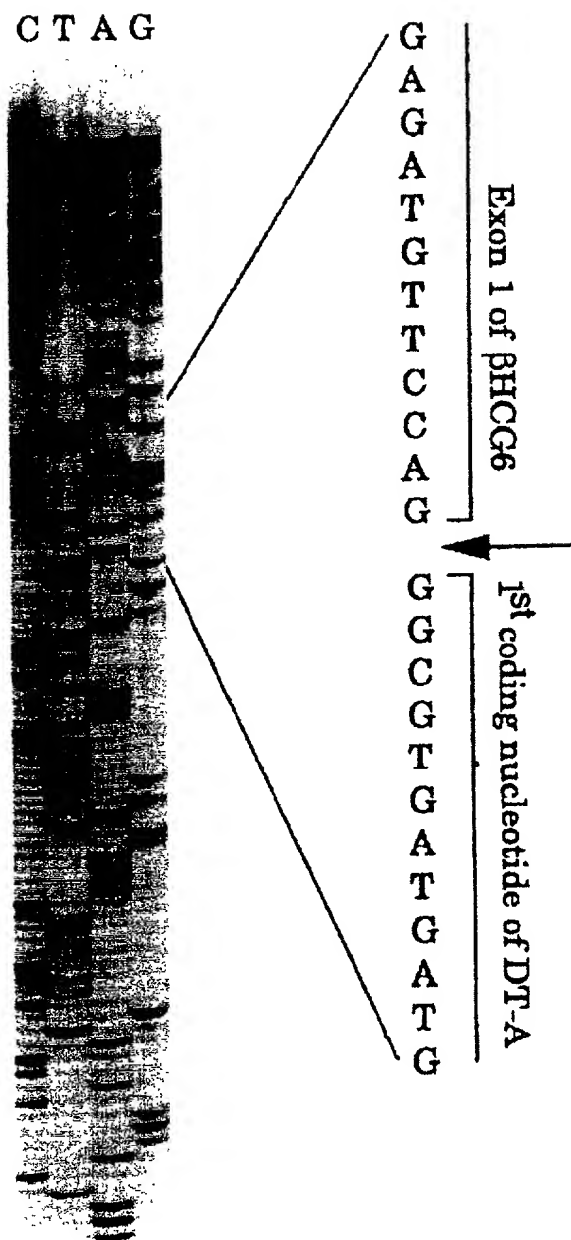
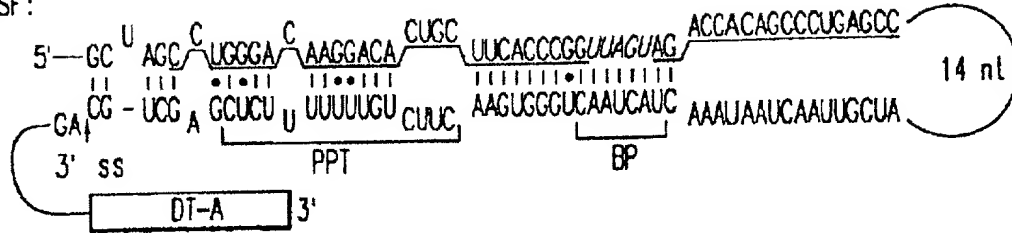


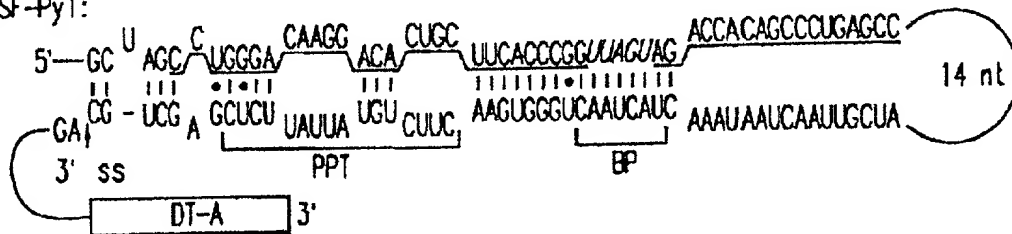
FIG.3

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1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

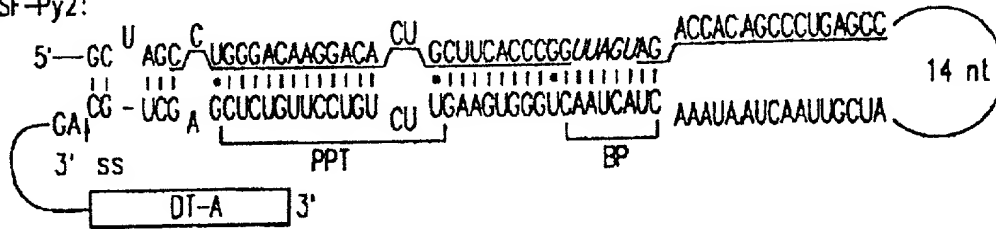


FIG.4A

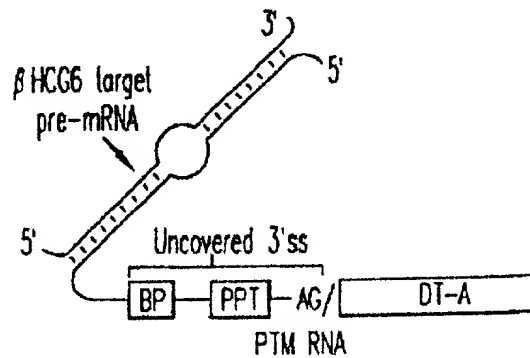


FIG.4B

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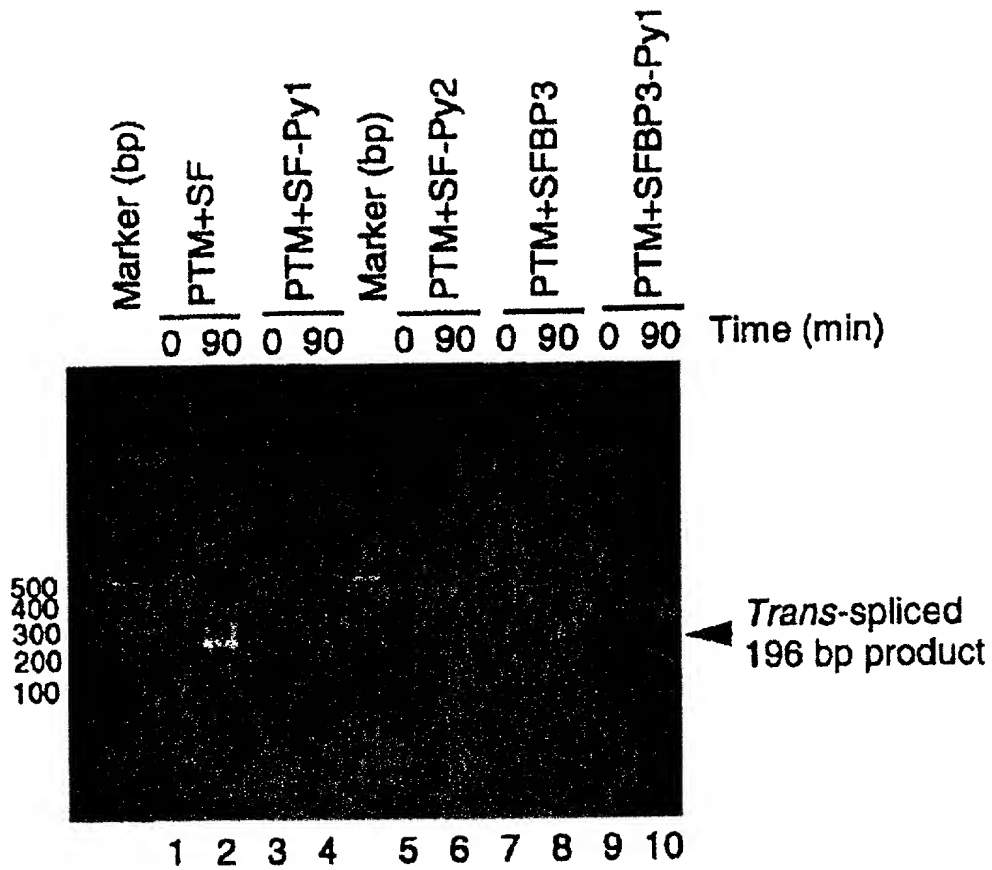
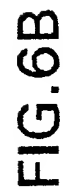
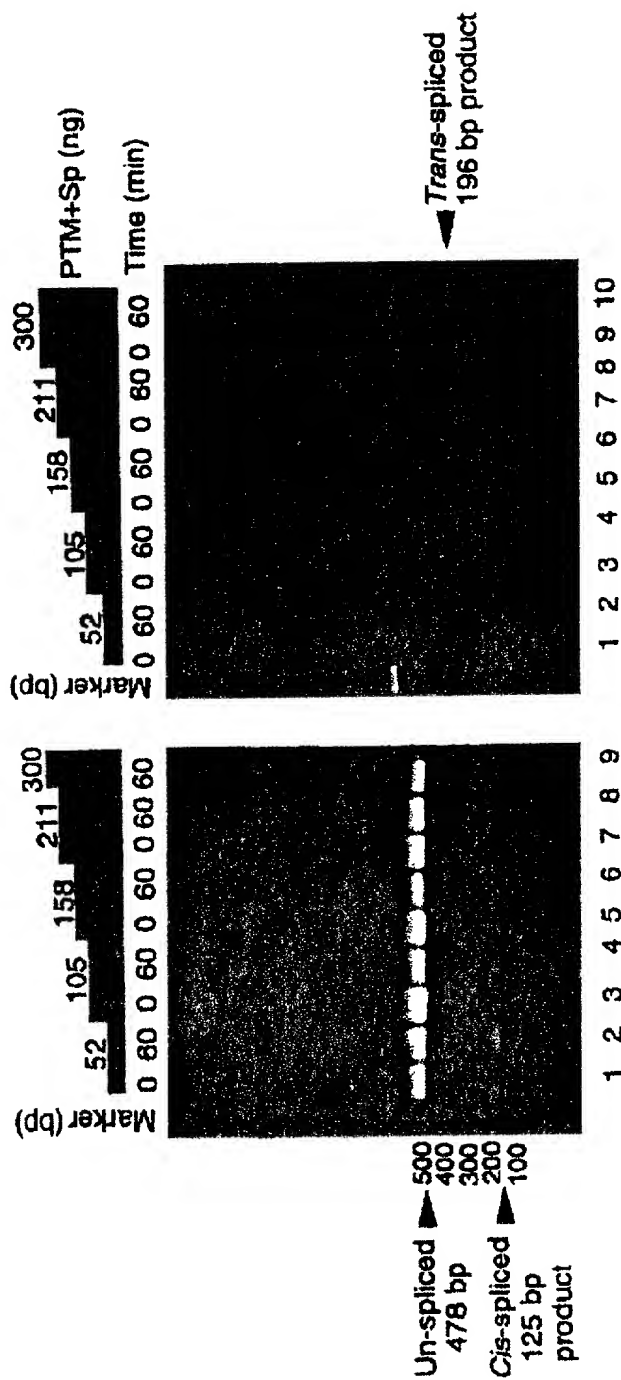


FIG.4C



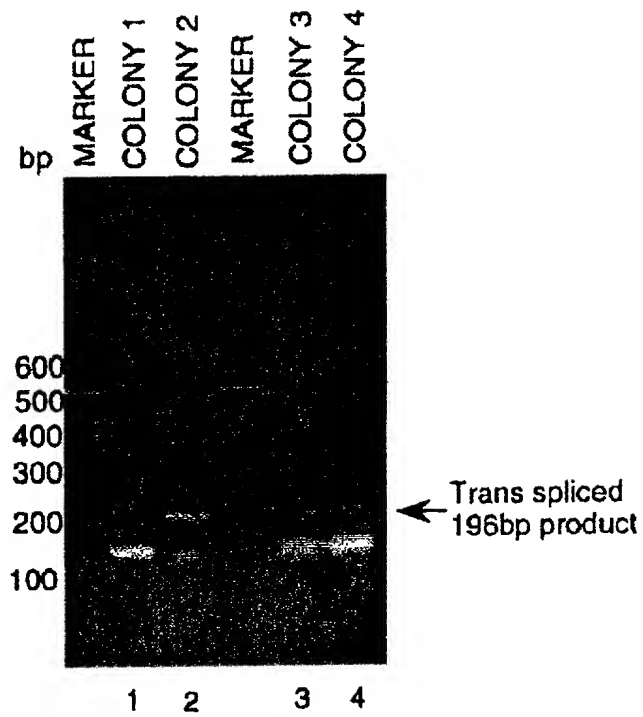


FIG.7A

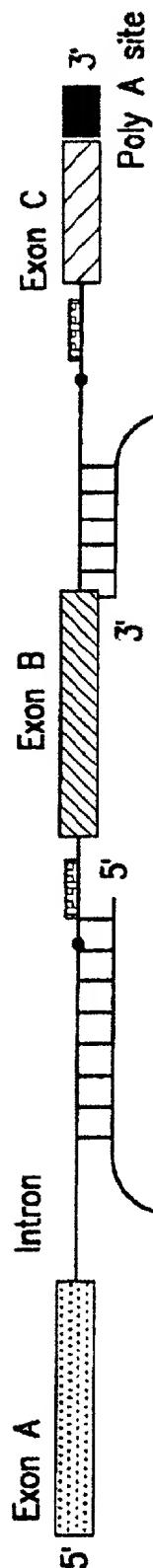
EXON 1 OF β HCG6 ↓
 5'-CAGGGACGCACCAAGGATGGAGATGTTCCAG-GGGCTGATGATGTTGTT
 ↓ 1ST CODING NUCLEOTIDE OF DT-A
 GATTCCTTAAATCTTTTGATGGAAACTTTCTTCGTACCAACGGGACTA
 AACCTGGTTATGTAGATTCCATTCAAAA-3'

11 8 91

FIG.7B

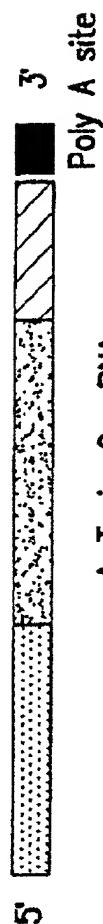


Cis-splicing



Double splicing pre-therapeutic RNA

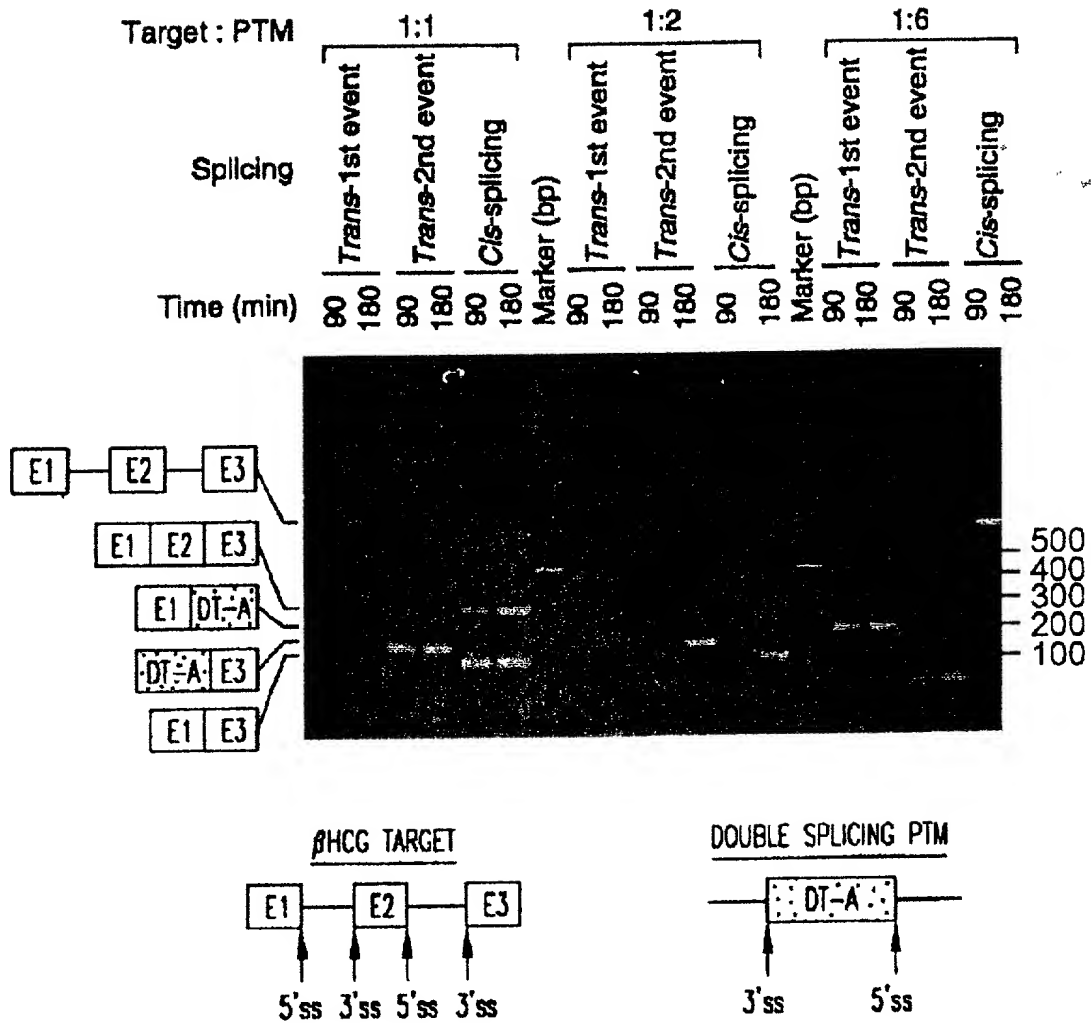
Trans-splicing



A-Toxin-C mRNA

FIG.8A

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Cis-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. *Trans*-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. *Trans*-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

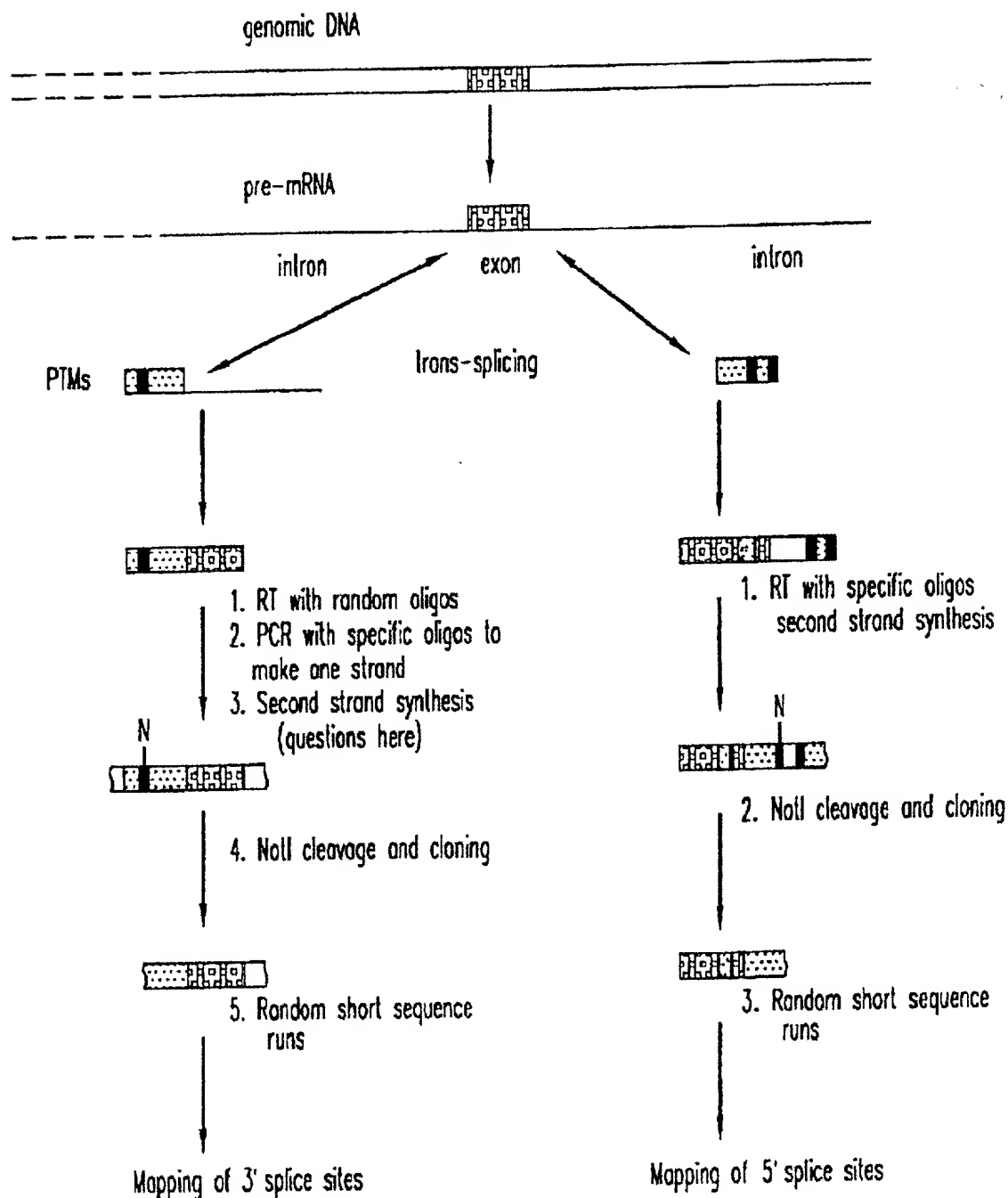
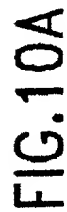


FIG.9



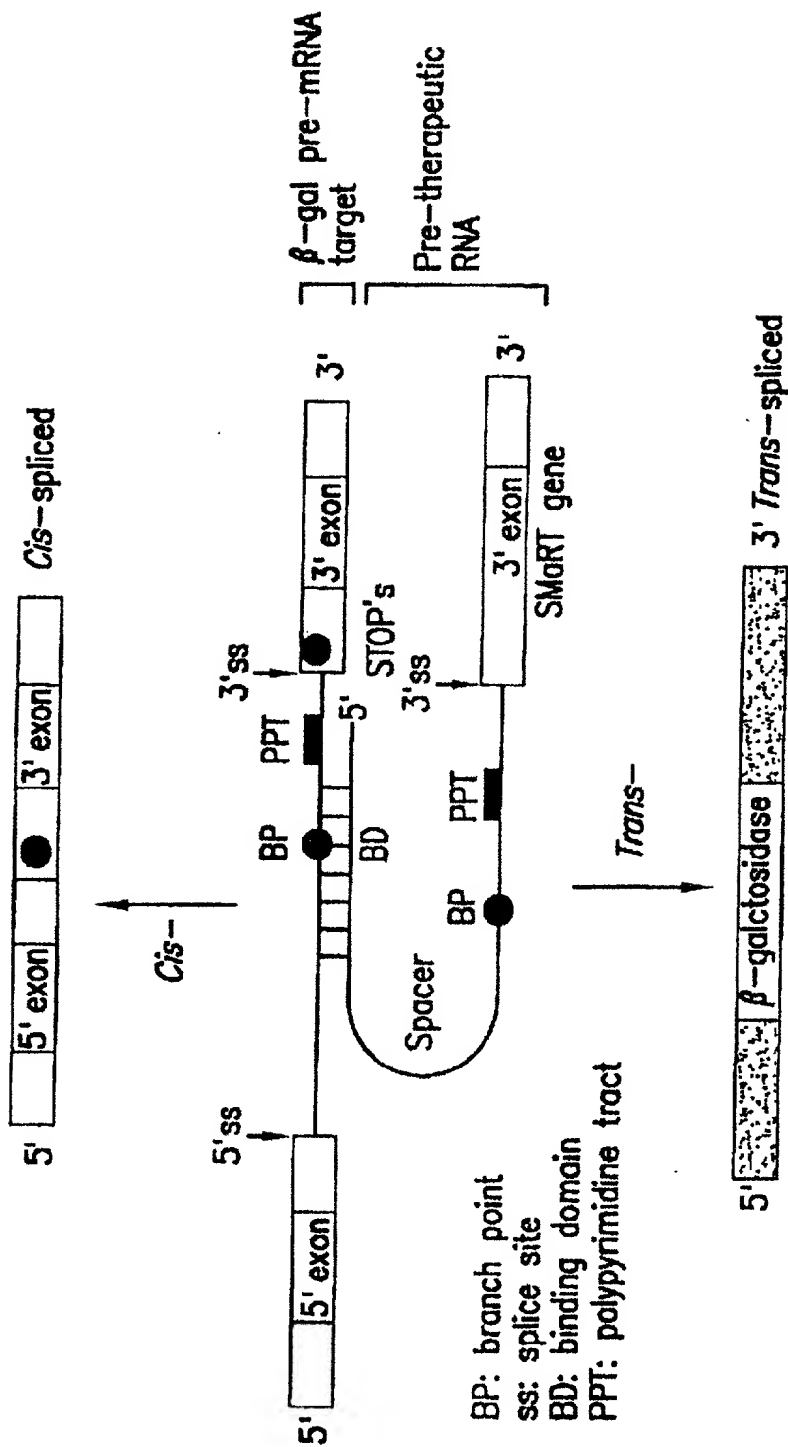


FIG. 10B

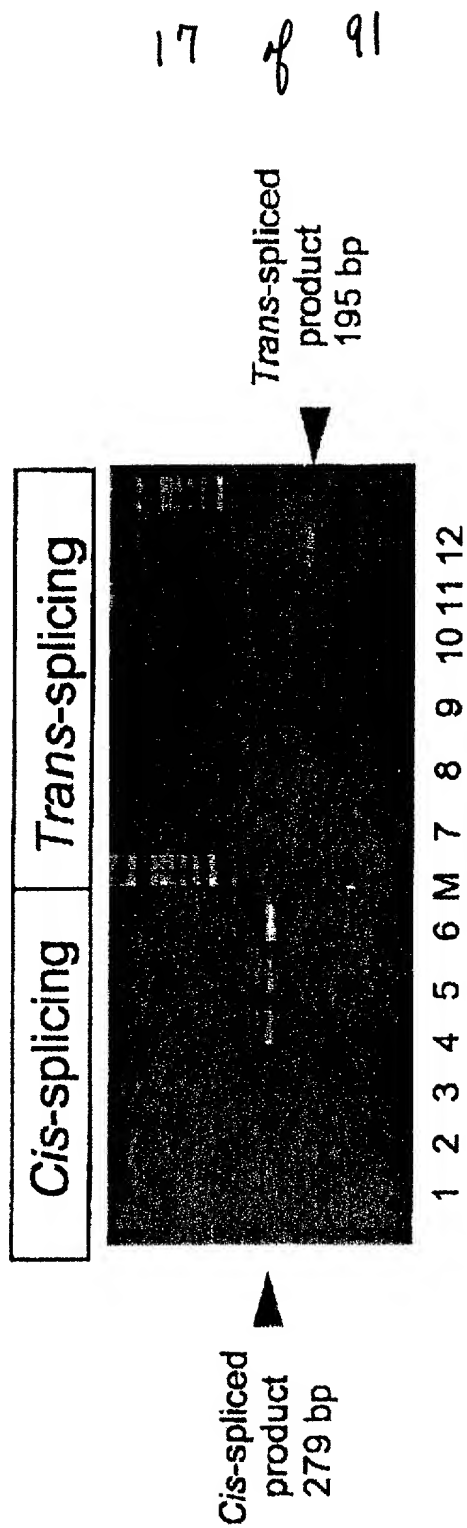


FIG.11A

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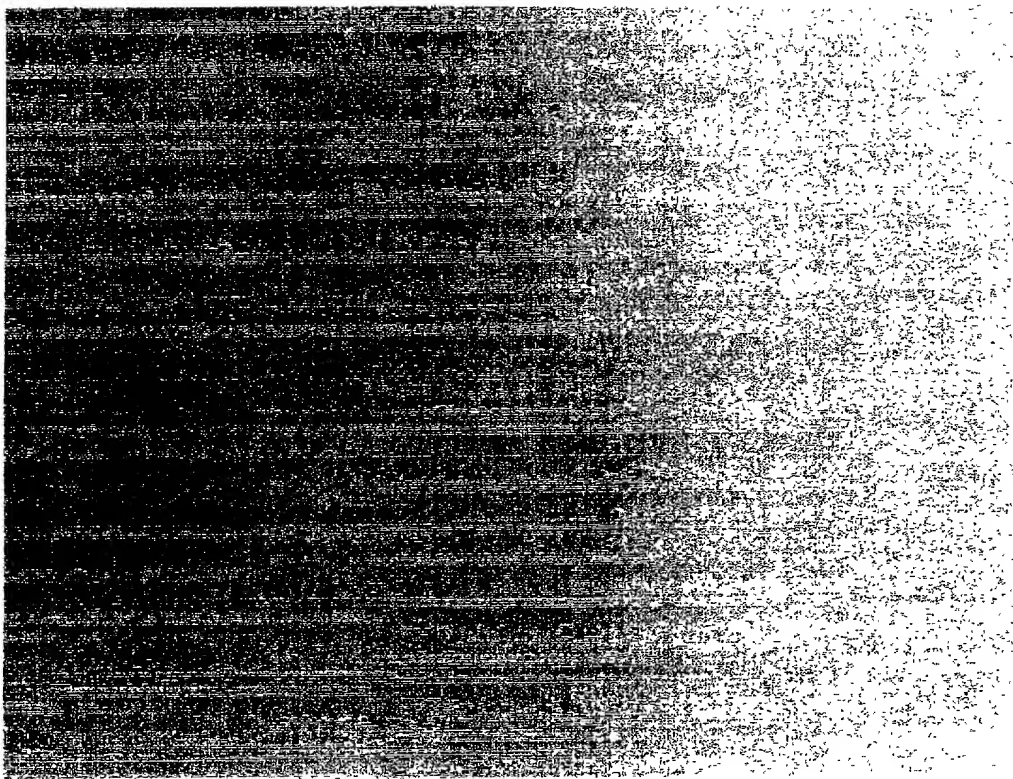


FIG.11B

204040" 254TH660

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20400254650

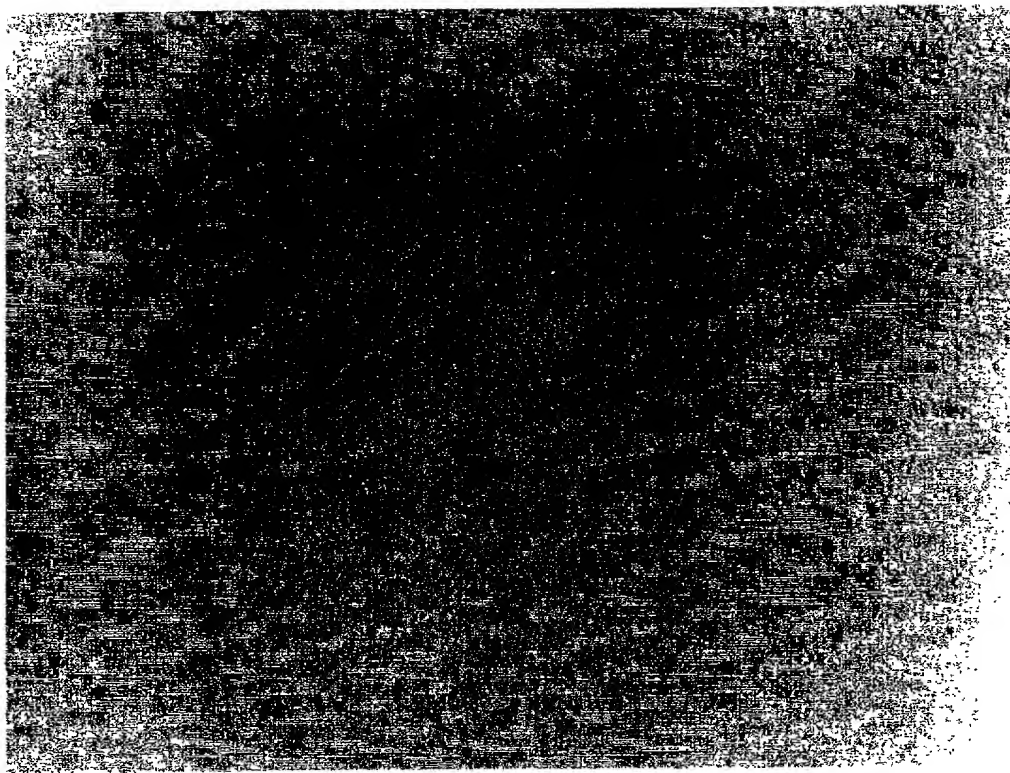


FIG.11C

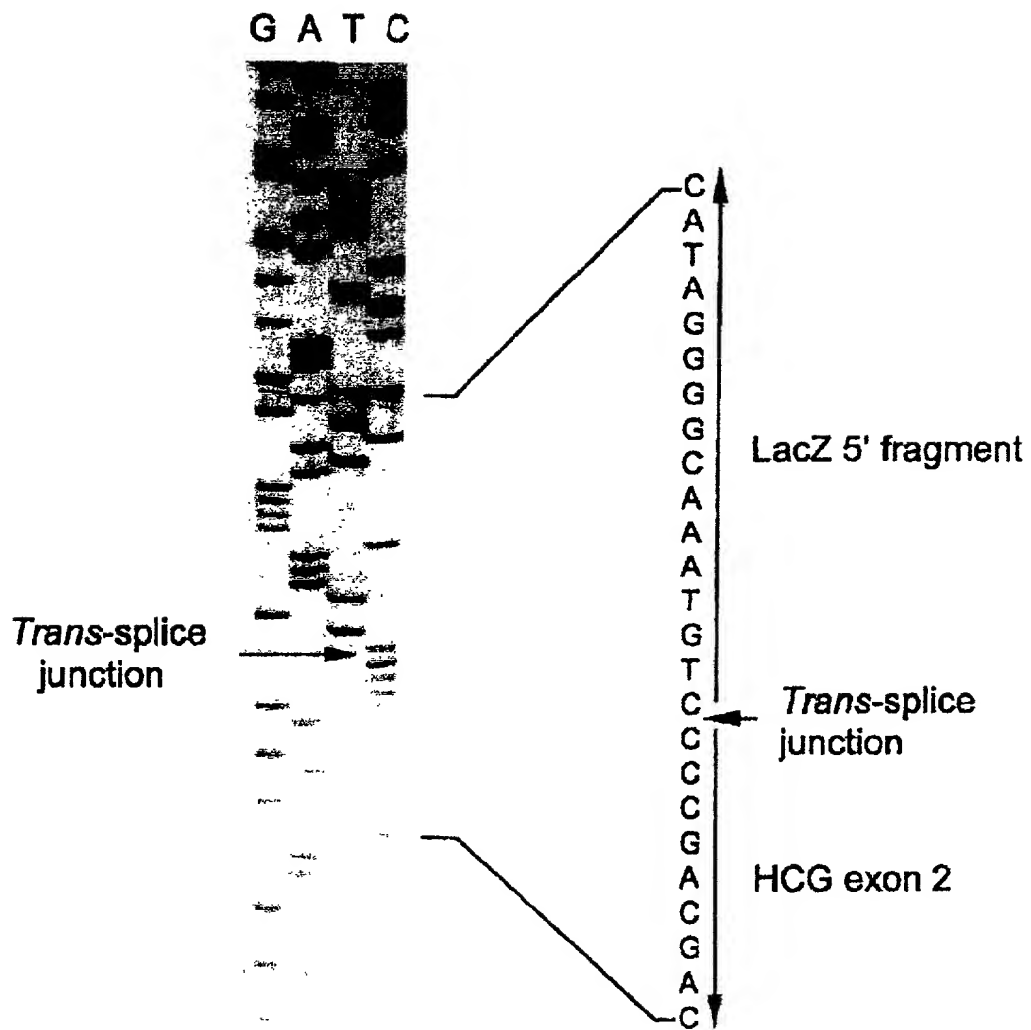


FIG.12A

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

Biolac-TR1

GGCTTTGGCTACCTGGAGAGACGGCCCGCTGATCCTTTGGGAATACGCCCAACGGCATGGGTAAACAGTCTTG

Splice junction

GGGGTTTCGCTAAATACTCGCAGCGTTTCGTCAGTATCCCGTTTACAG/GGCGGCTTCGTCTAATAATG

GGACTCGGTGGATCAGTCGCTGATTAAATATGATGAAACGGCAACCGTGGTCGGCTTACGGGGGTGATTT

Lac-TR2
TGGCGATACGCCGAACGATCGCCAGTTCCTCTATGAACGGTCTGGTCTTTTGGCGACGGCAAGGCGCATCCAG

Lac-TR2

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

Biolac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTTGGCAATACGCCCAACGGATGGGTACACAGTCTTGG

Splice junction

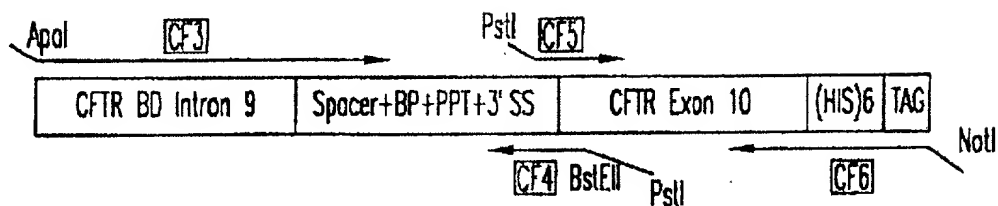
CGGTTTCGGCTAAATACITCGCAGCGTTTCGTCAGTATCCCGTTTACAG/CGGCTGCTGCTGTTGCTGCTGCT

HCCR2

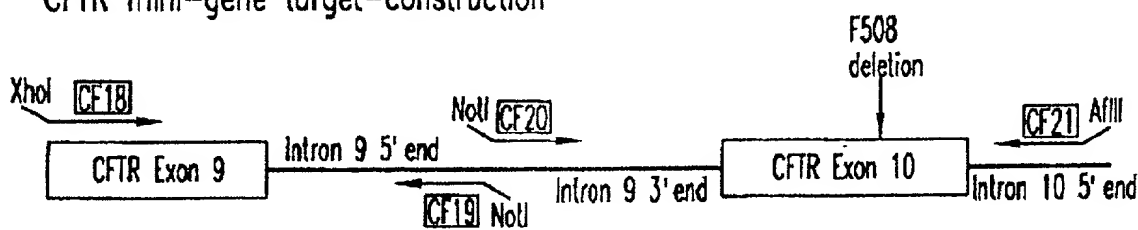
GAGCATGGCGCGACATGGGCATCCAGGAGCCACTTCGGCCACGGTGGCG

FIG. 12B

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

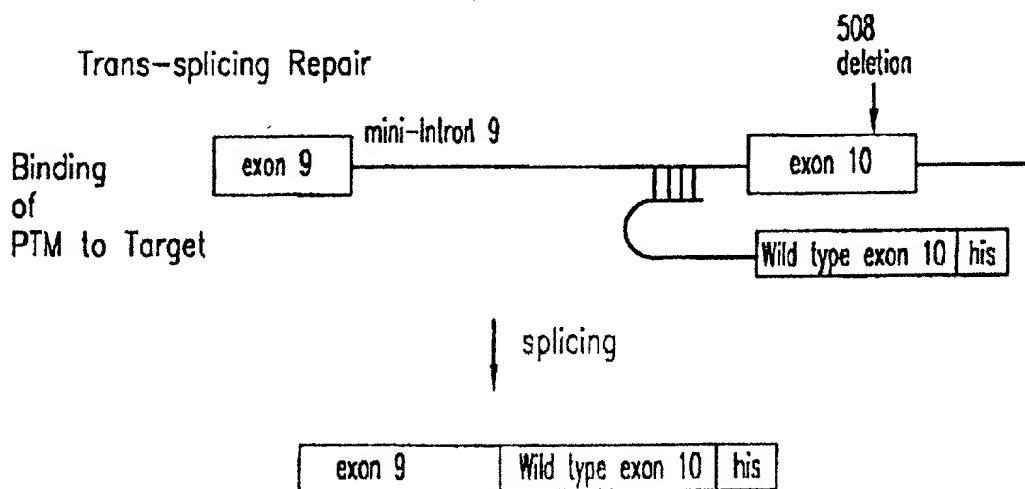


FIG.13

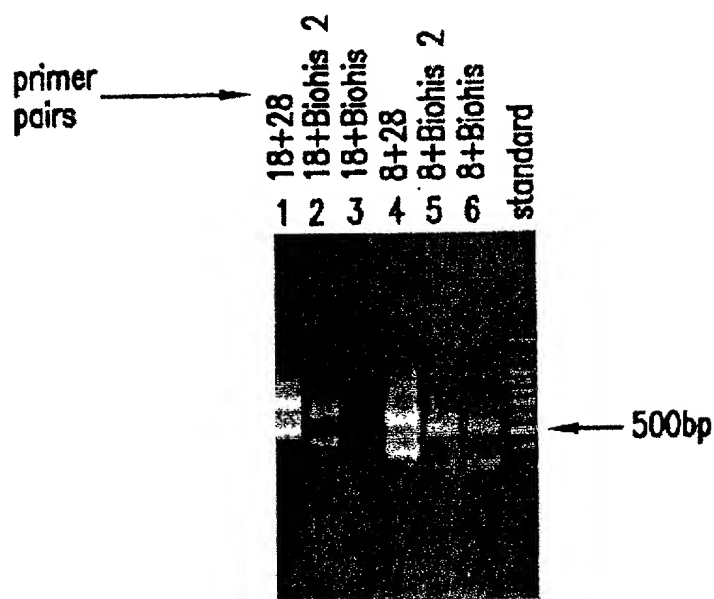


FIG.14

Positions of Restriction Endonucleases sites (unique sites underlined)

Kpn I Pst I EXON 10 CFTR + HIS TAG + STOP
 1 1 3'ss
 160
 TGGTACCTCTCTCTTTTCTCGCAGACTTCACCTCTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGCGTAAAT
 ACCATGGAGAGAGAAAAAAGGAGCTCTGAAGTGAAGATTACTACTAATACCCCTCTTGACCTCGGAAGTCTCCCATTTTA
 82 102
 Dde I
 Xmn I
 172 190 Sph I HIS
 240
 TAAGCACAGTGGAGAAATTCATCTGTTCTCAGTTTCTCGGATTATGCCTGGCACCATTAAAGAAAAATATCATCTTTG
 ATTCGTGTCACCTTCTTAAAGTAAGACAGAGTCAAAAGGACCTAAIACGGACCGTGGTAATTTCTTTTATAGTAGAAAC
 STOP
 320
 GTGTTTCCTATGATGAATATAGATACAGAAGCGTCATCAAAGCATGCCAACTAGCAAGCATCATCATCATCATATAG
 CACAAAGGATACTACTTATATCTATGCTTCGCAGTAGTTTCGTACGGTTGATCTTCTCGTAGTAGTAGTAGTAATC
 282

FIG. 15A

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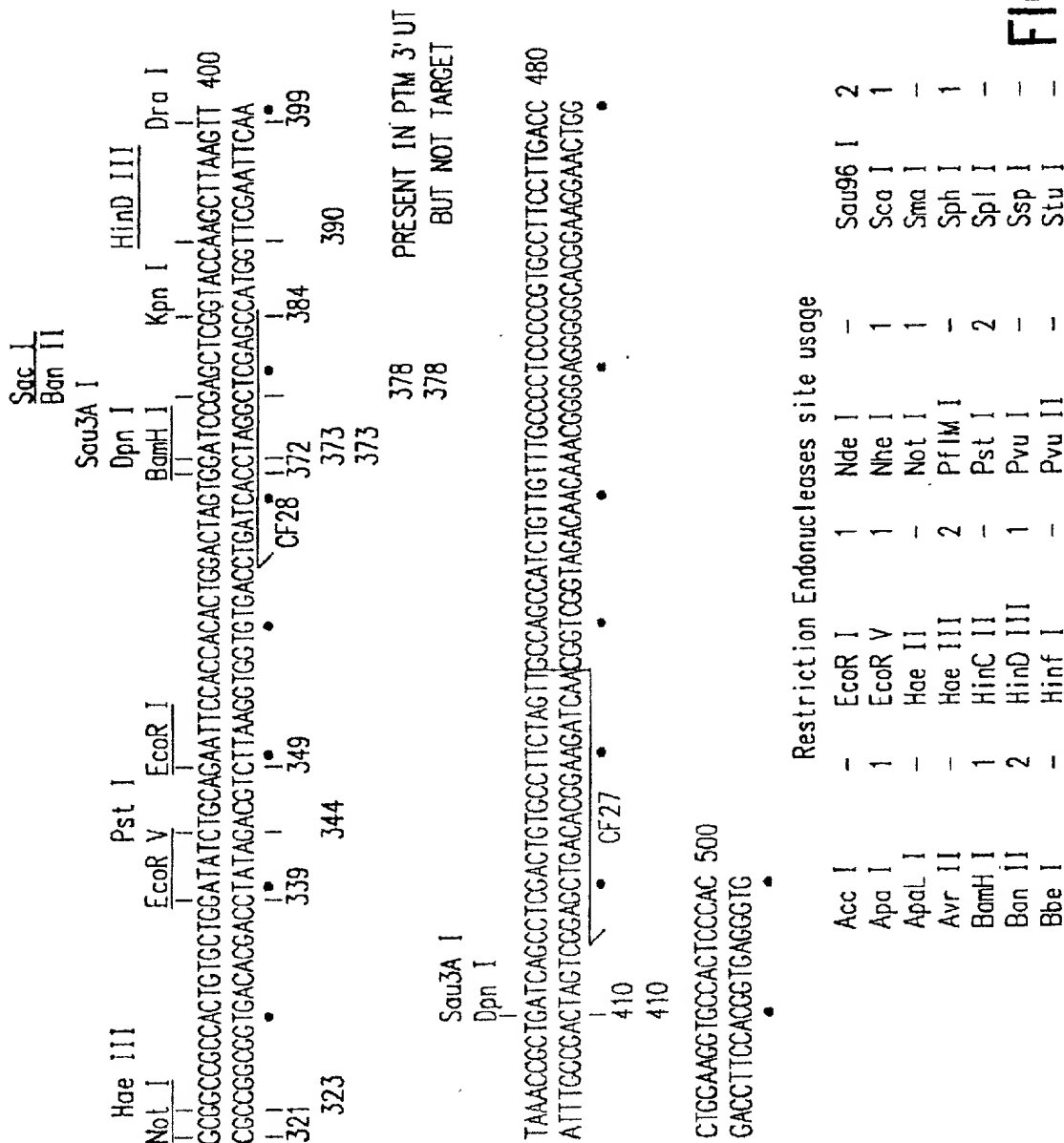
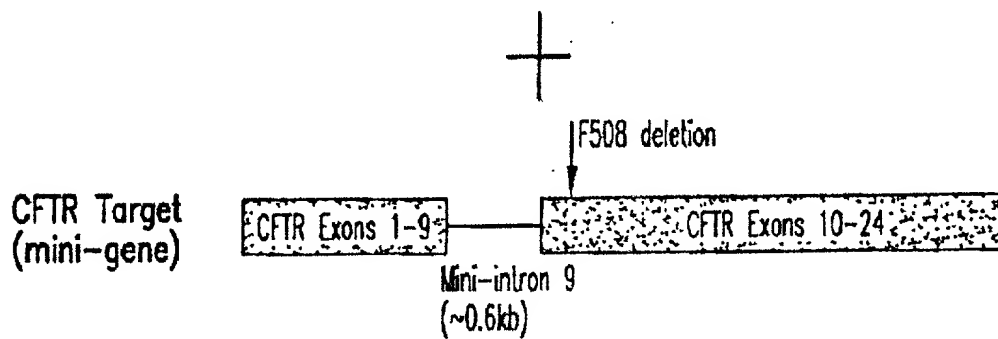
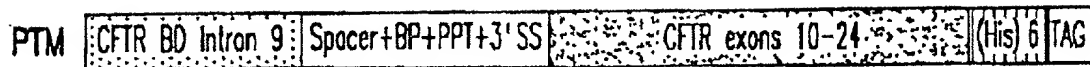


FIG.15B

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Cotransfect PTM and target molecules in HEK 293 cells and detect repaired CFTR mRNA by RT-PCR.

Repaired CFTR mRNA

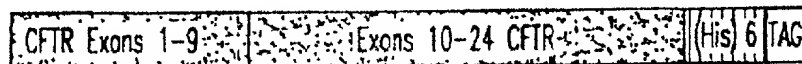


FIG.16

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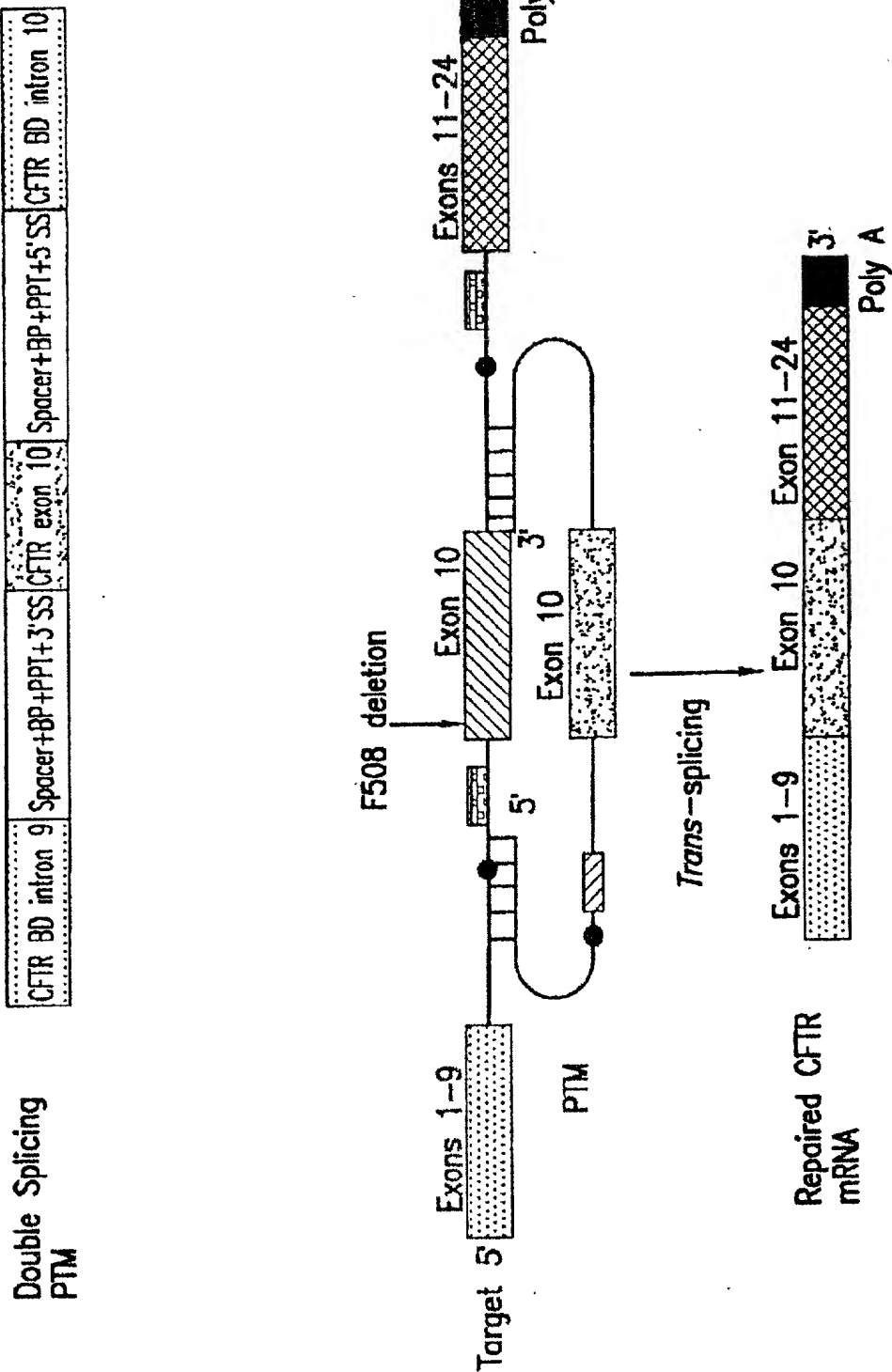


FIG.17

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DOUBLE TRANS-SPLICING SPECIFIC TARGET

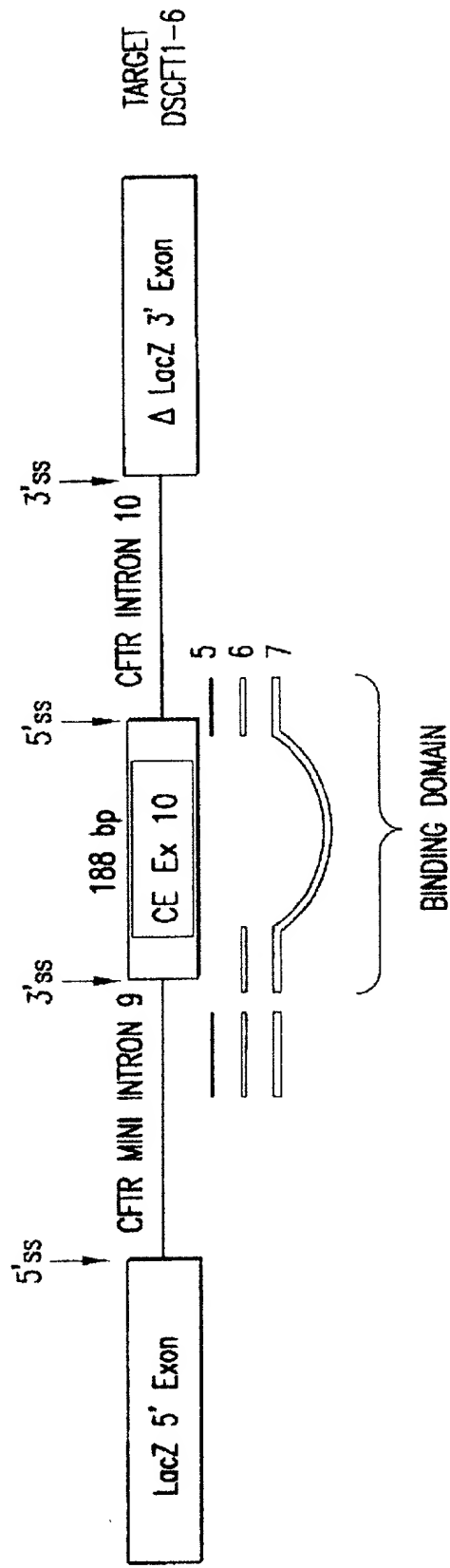
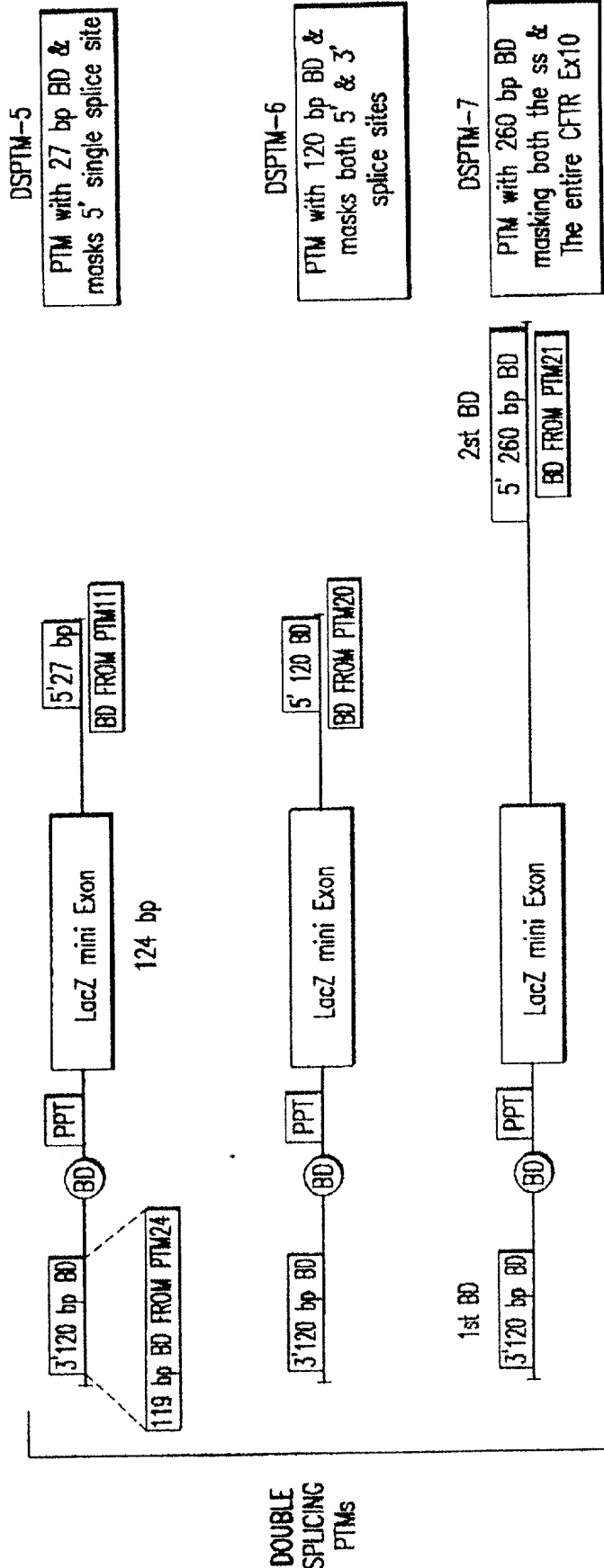


FIG.18

29 2 91

DOUBLE TRANS-SPLICING PTMS



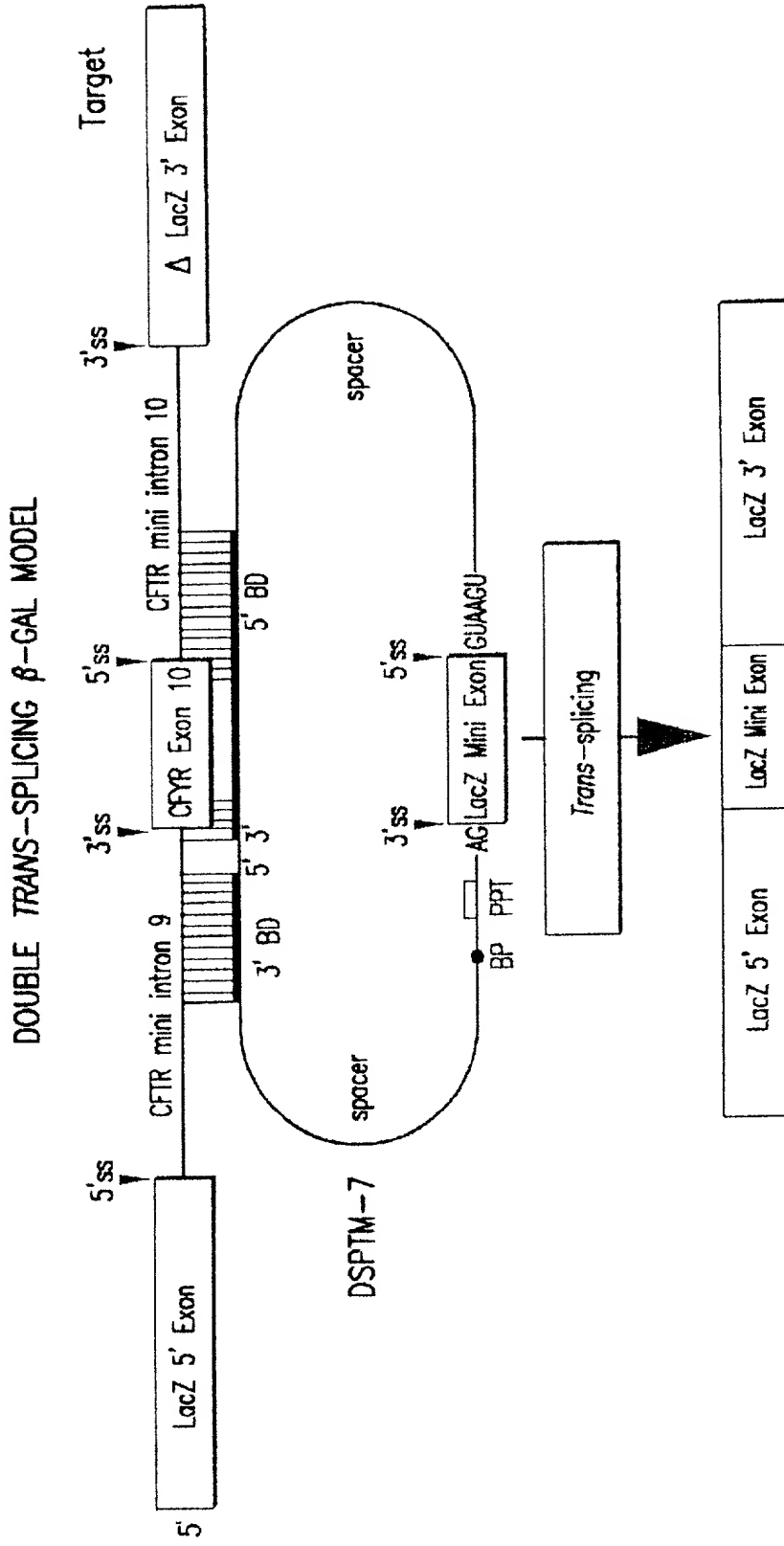
DSPTM-5
PTM with 27 bp BD &
masks 5' single splice site

DSPTM-6
PTM with 120 bp BD &
masks both 5' & 3'
splice sites

DSPTM-7
PTM with 260 bp BD
masking both the ss &
The entire CFTR Ex10

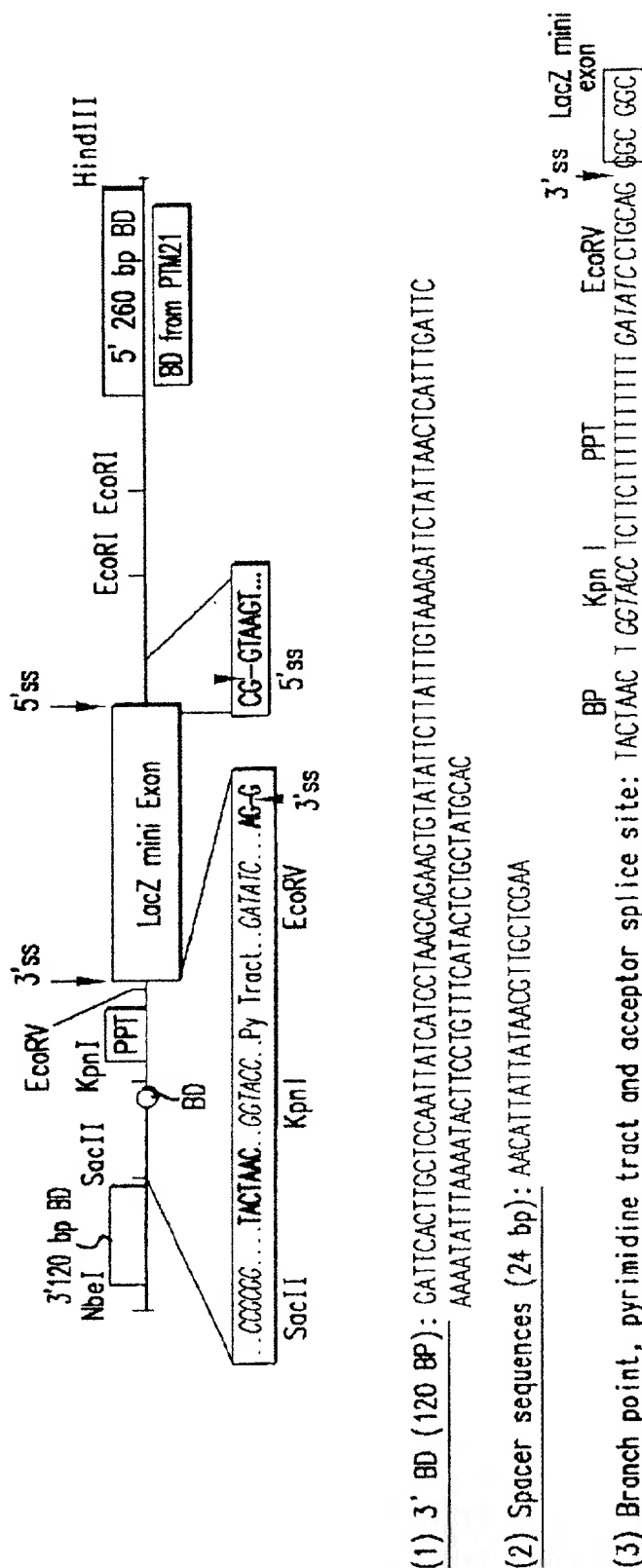
FIG.19

DOUBLE
SPlicing
PTMs



Repaired LacZ mRNA

FIG.20



(1) 3' BD (120 BP): GATTCACITGGCICCAATATCATCTTAAGCAGAGCTATATCTTATTGTGAAGATCTATTAACATCATTCGATTC
AAAAATTTAAATACTCTCGTTTCATCTCGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTIGCTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site: TACIAAC TGGTACC TCTCTCTTTTTTTT GATATCTCTGCAG GGC GGC

(4) 5' donor site and 2nd spacer sequence:

LacZ mini 5' ss
exon ↓

TGA ACC G T A A G T G T T A T C A C C G A T A T G T C T A A C C T G A T T C G G S C C T T C G A T A C G

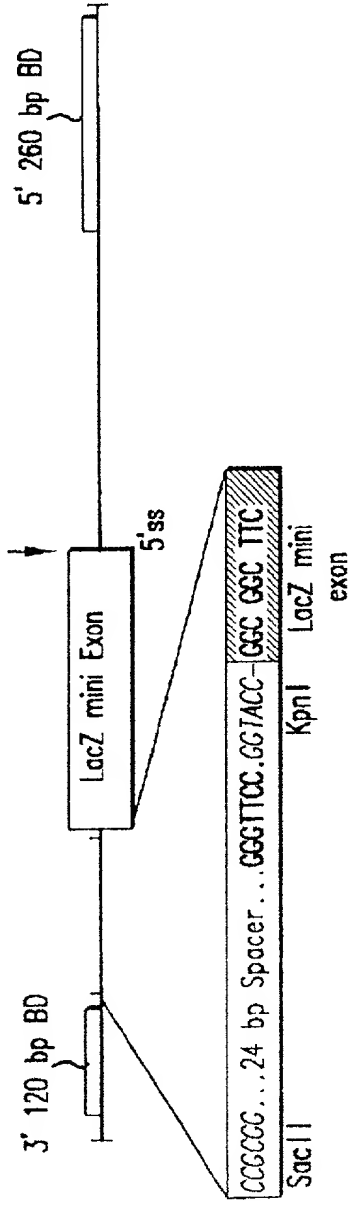
C T A A G A T C C A C C G G

(5) 5' BD (260 BP): TCAAAAAGTTTTTCACATAATTTCTTACCTCTCTCTTGAA/TCATGCTTTTCATGACCGCTTCGTATCTAATTCATCATTCGAA
ACACCAATGATTTTCTTTTAATGTCGCTGGCATATCTCGAAAACTGATAACACAA/TCGAAATTTCTCCACTGTGCTTAA
AAAAACCGCTCTGAA/TC/CCATTTCTCCCATATATCATCTATGAACTGAACCTCTGGAAATAAACCATCATTTATTAACTCA
TTATCAAAATCAGGC

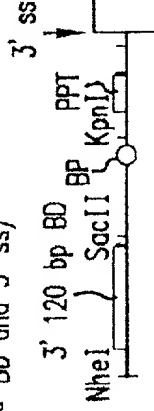
FIG. 21

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DSPTM8: (Δ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)

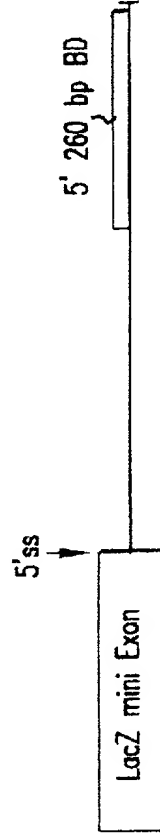


FIG.22

Mutants

ACCURACY OF DOUBLE TRANS-SPICING REACTION

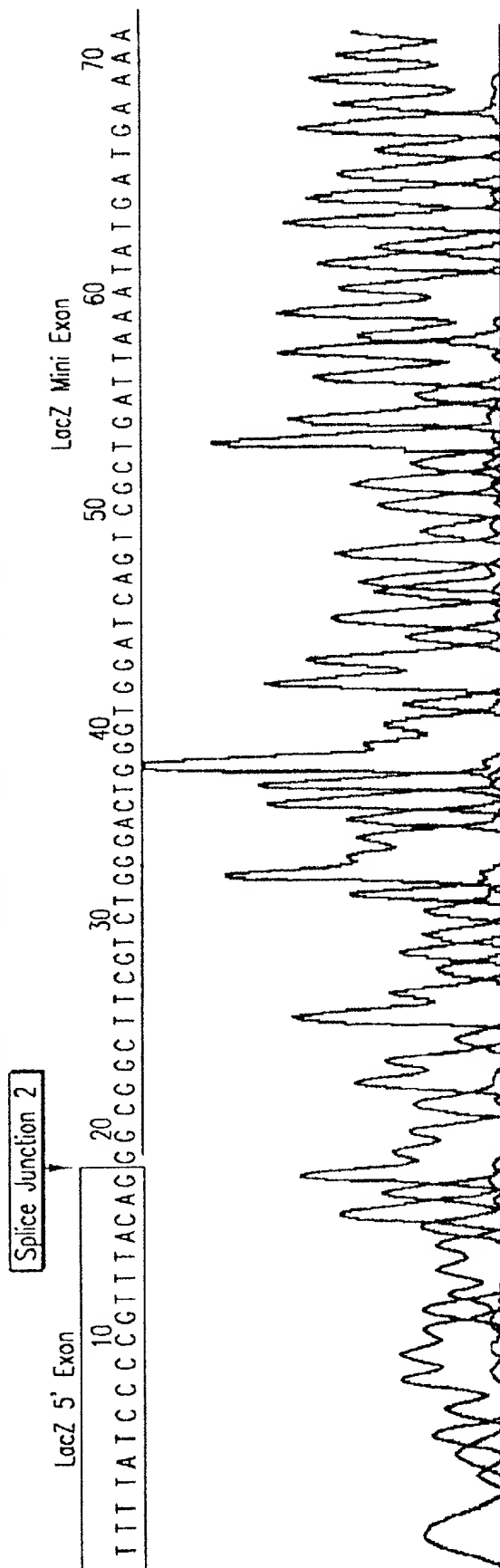


FIG.23A

ACCURACY OF DOUBLE TRANS-SPLICING REACTION

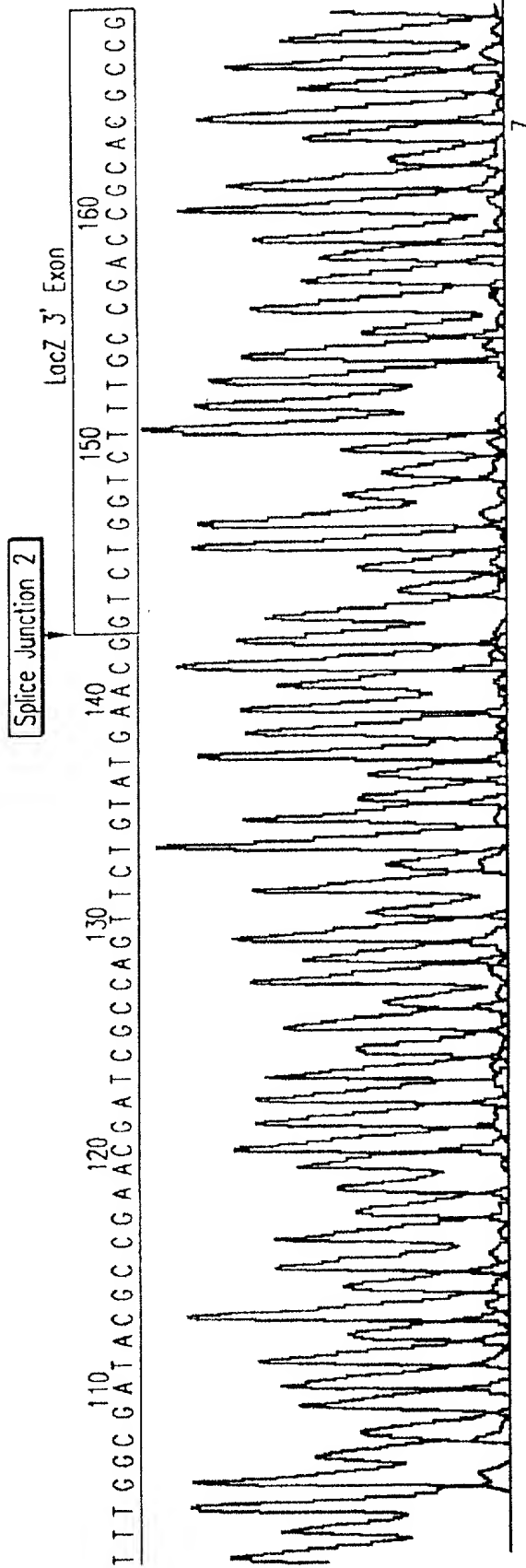


FIG.23B

Double Trans-splicing Produces Full-length Protein

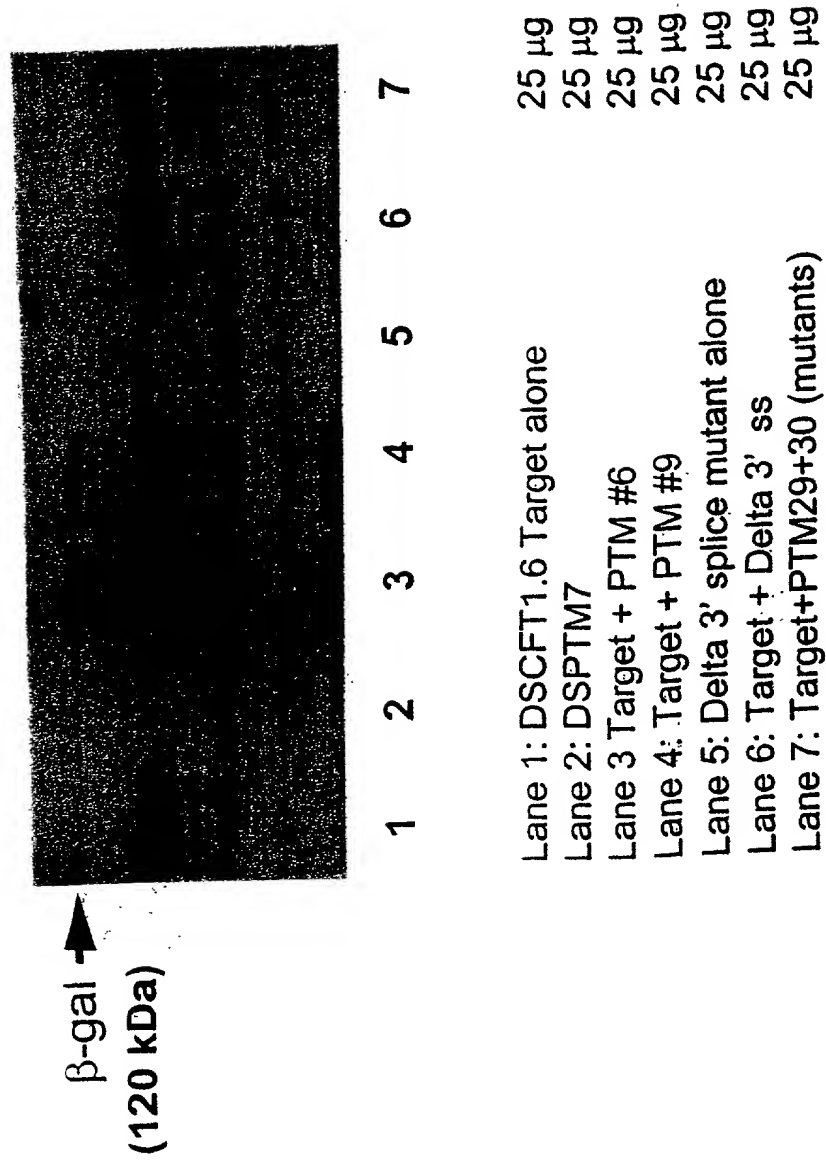


Figure 24

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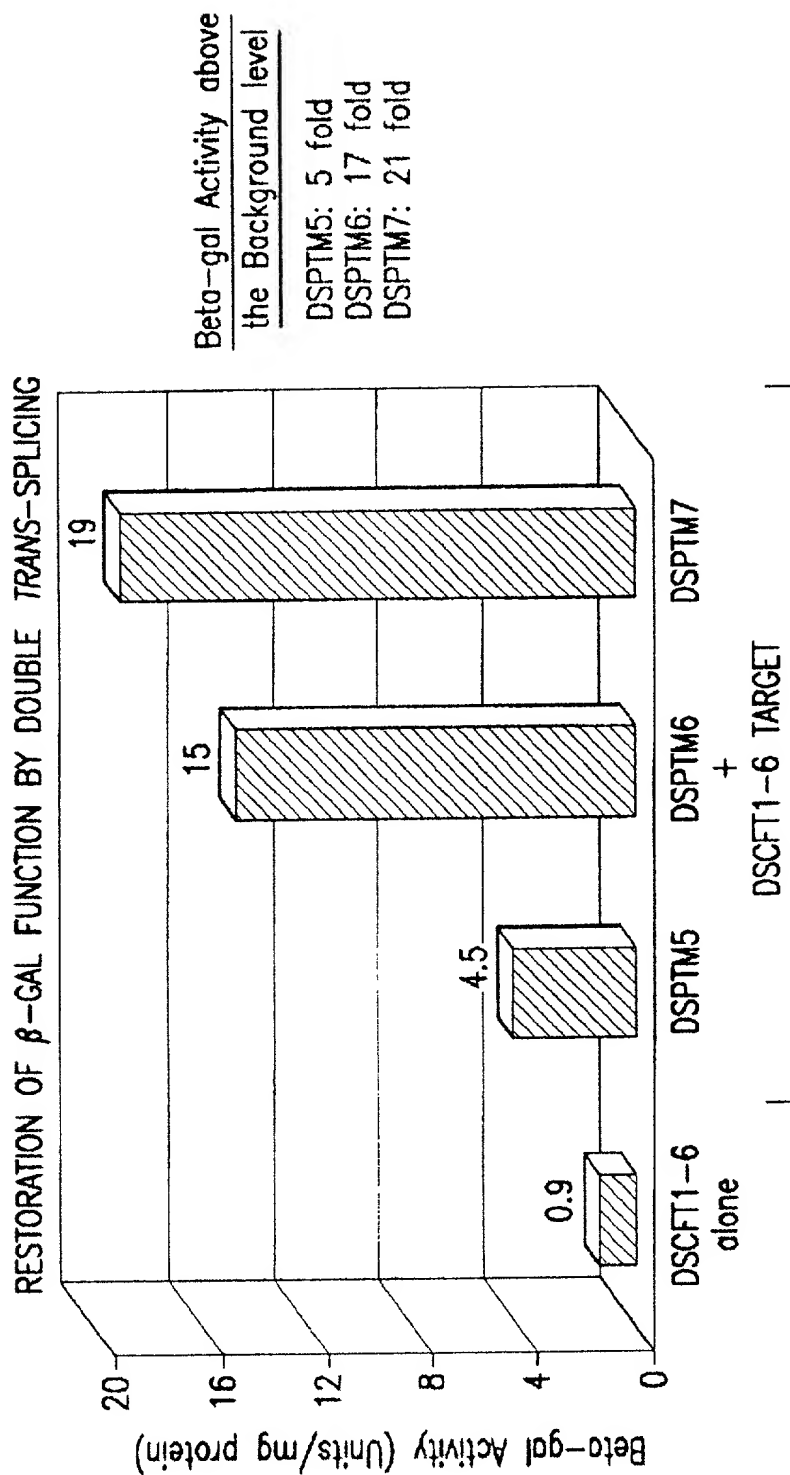


FIG.25

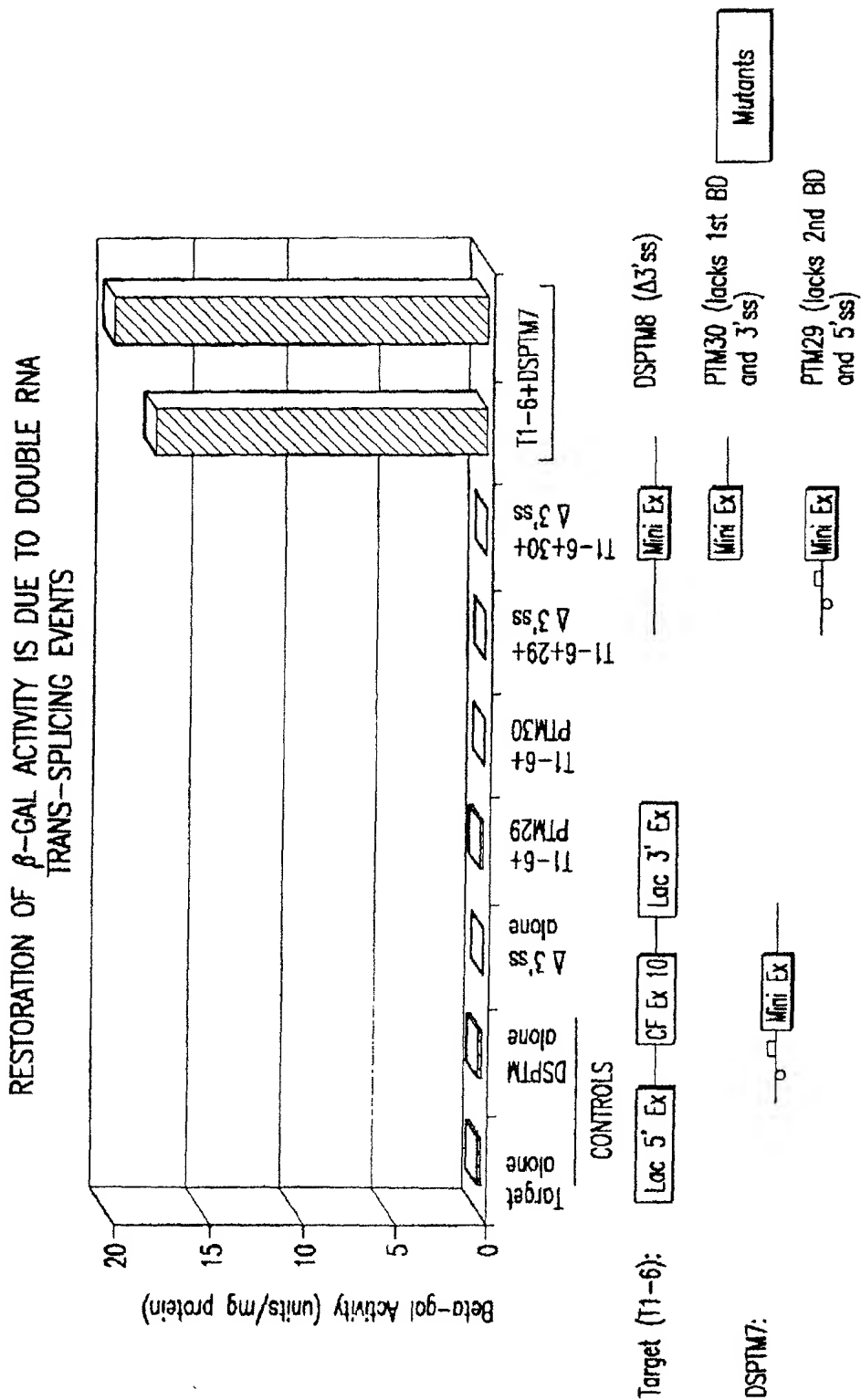


FIG.26

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The current level of beta-gal activity due to double trans-splicing is ~ 1-1.5% of the best single splice model (3' exon replacement)

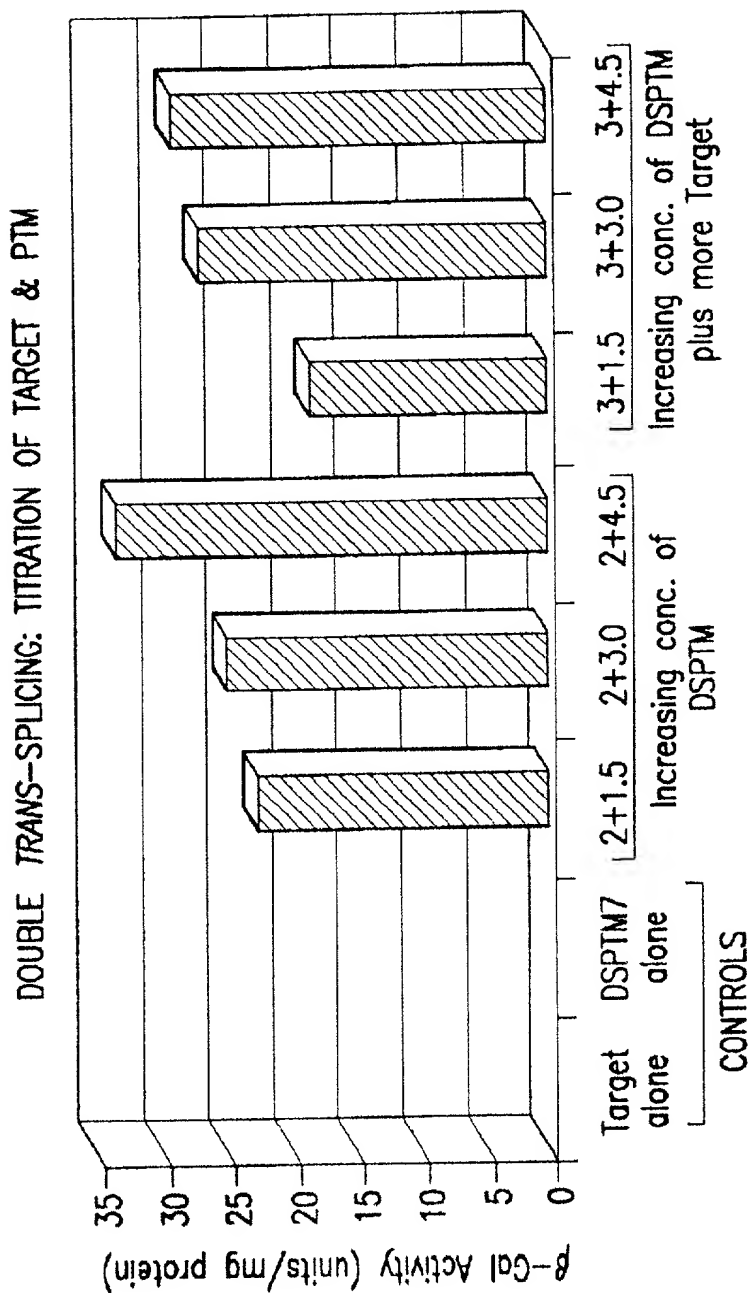


FIG.27

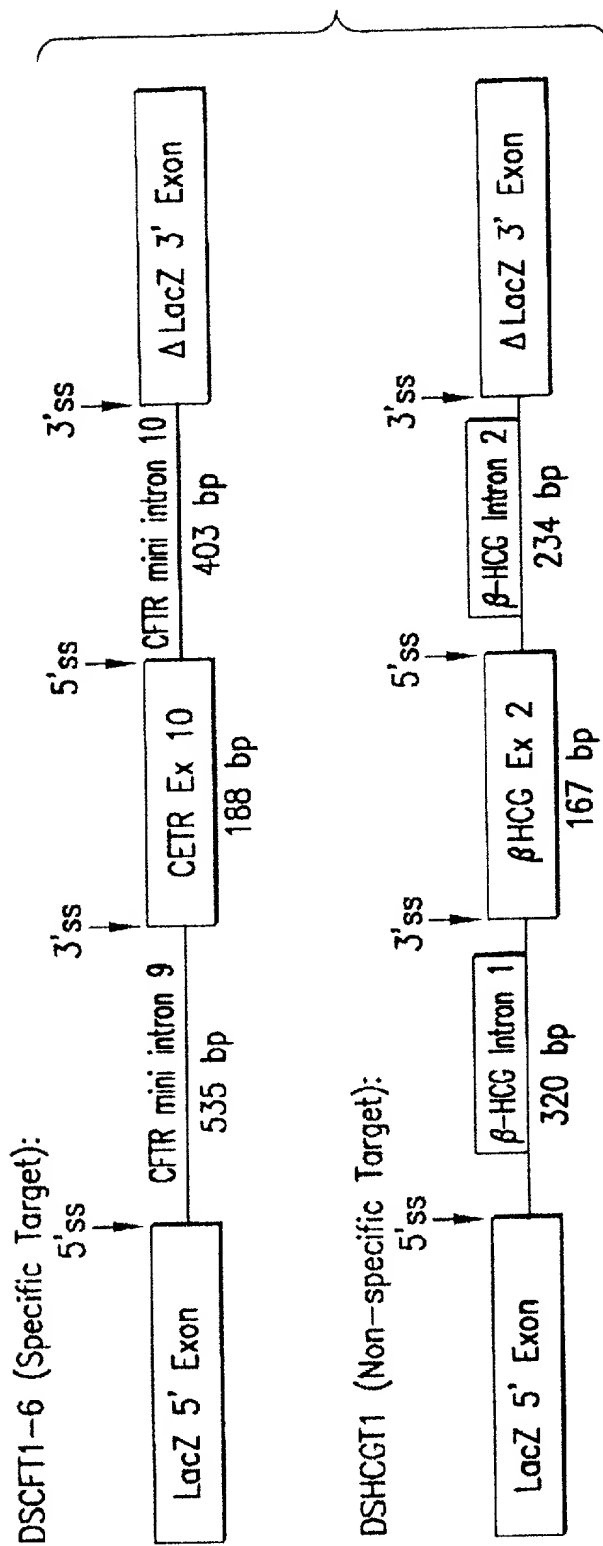


FIG.28

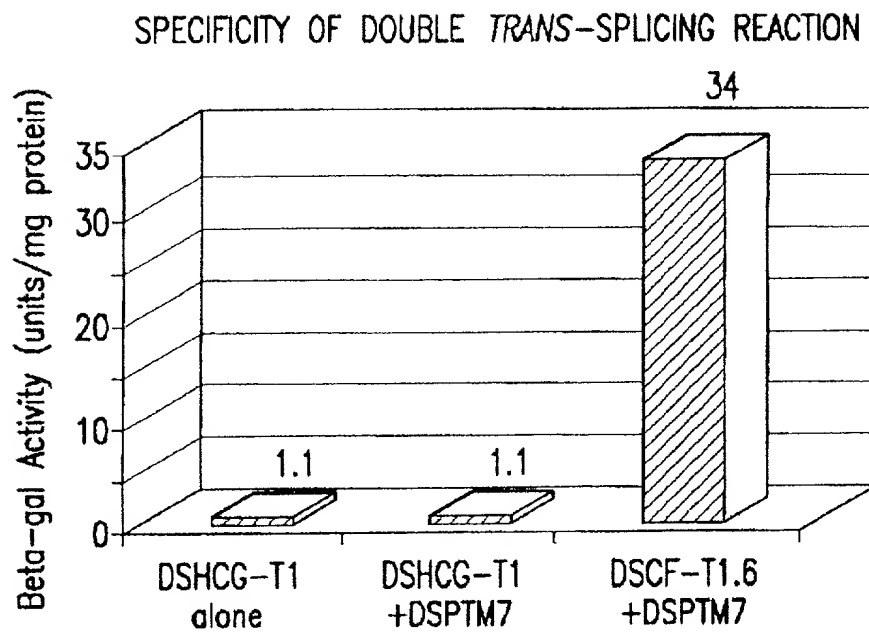
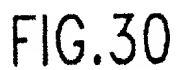
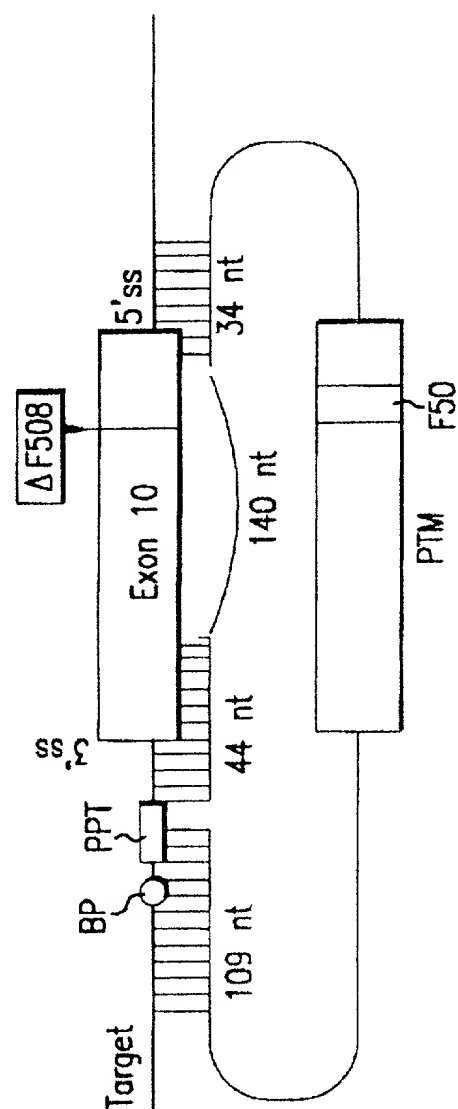


FIG.29



42 8 91

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGCTCATGATGATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCGG
GCGCATCAGCTTTTCAGGCCAATTCAGTGGATCATGCCCGTACCATCAAGGAGAACATAT
CTTCGCGCTCAGTACGACGAGTACCGCTATCGCTCGGCTGATTAAGCCCTGTCAGTTCGAGGAG

MCU in exon 10 of PTM
88 OF 192 (46%) bases in PTM exon 10 are not complementary to
its binding domain (bold and underlined).

FIG.31

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Sequence of a double Trans-spliced product

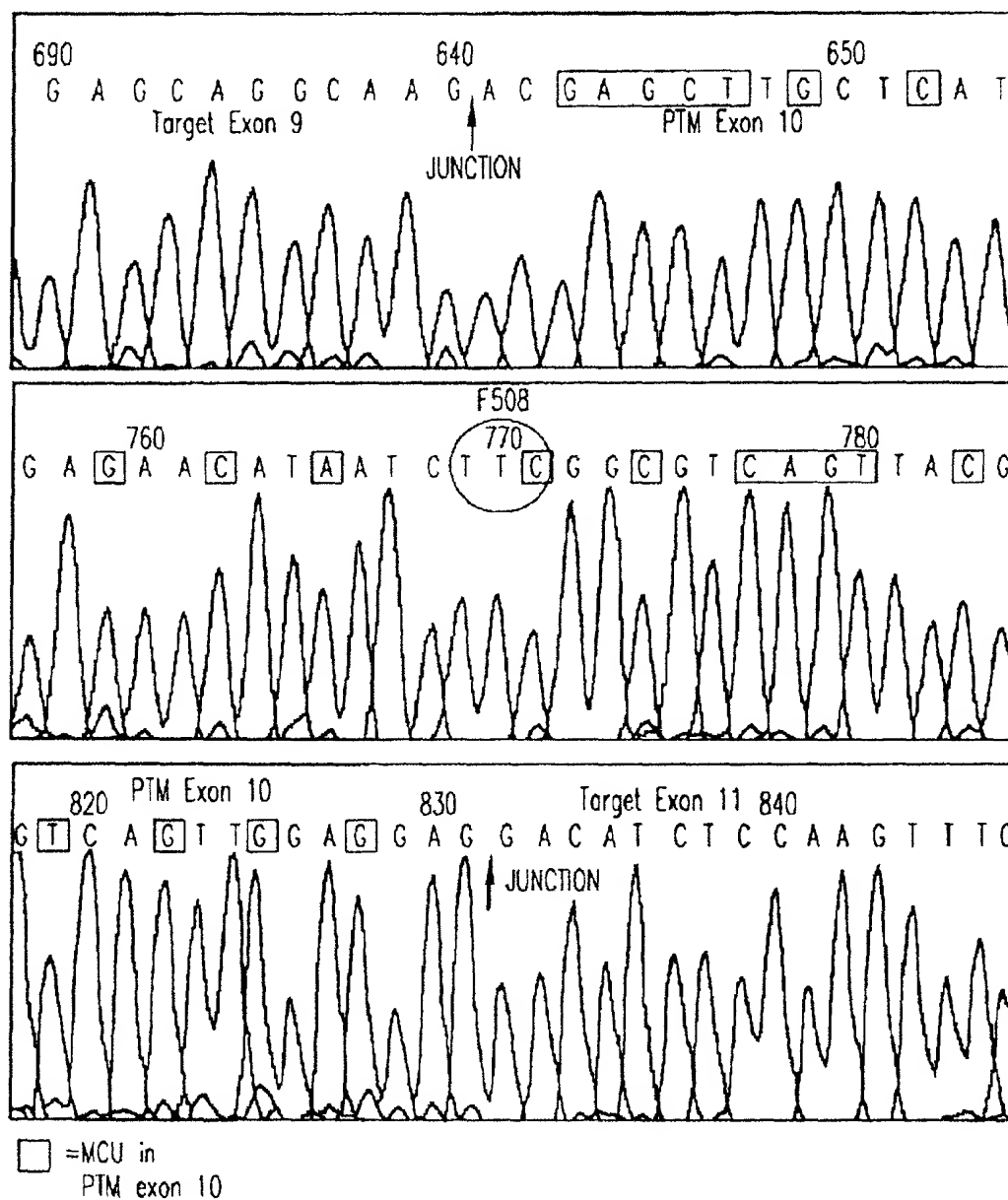


FIG.32

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CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

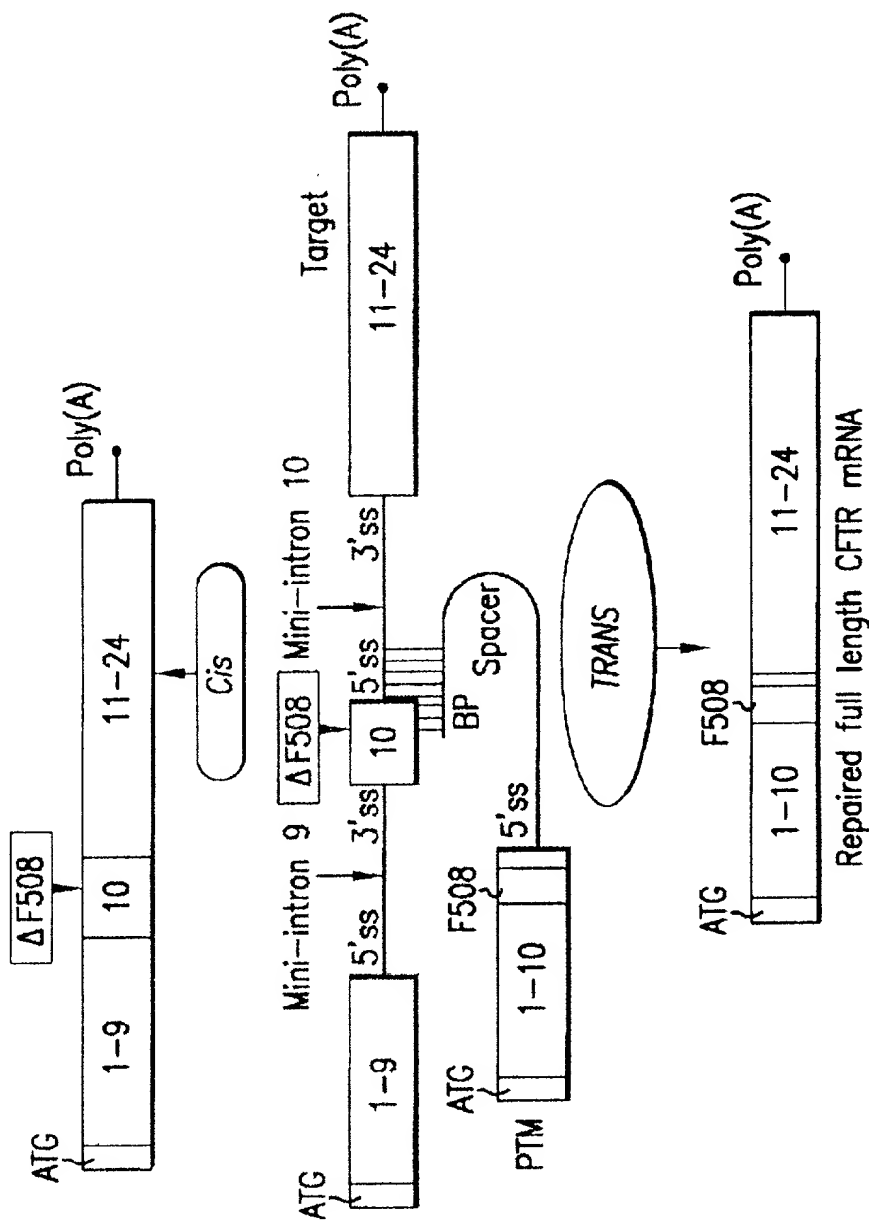


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

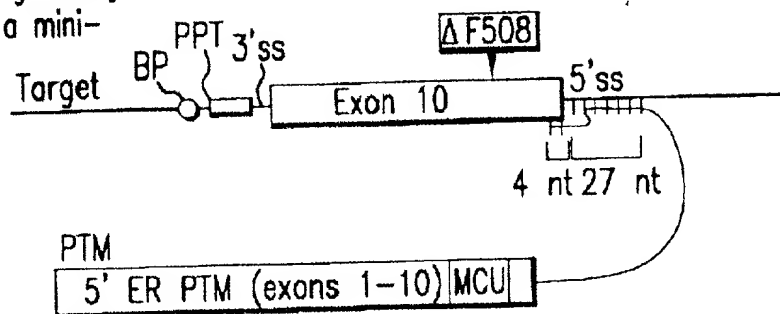


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

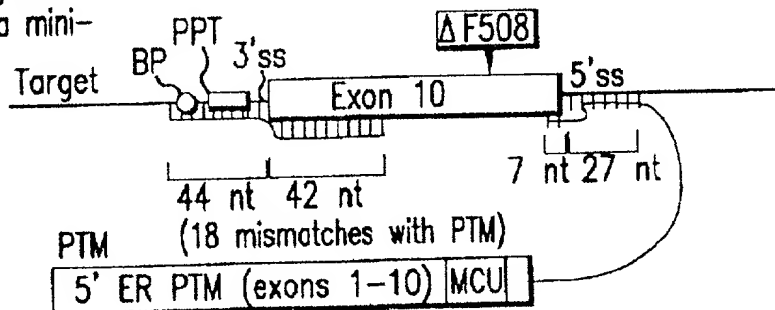


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

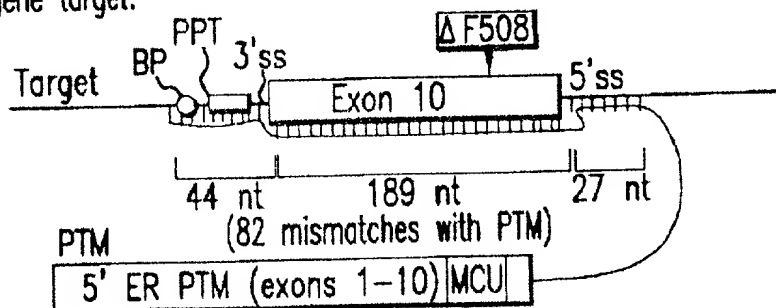
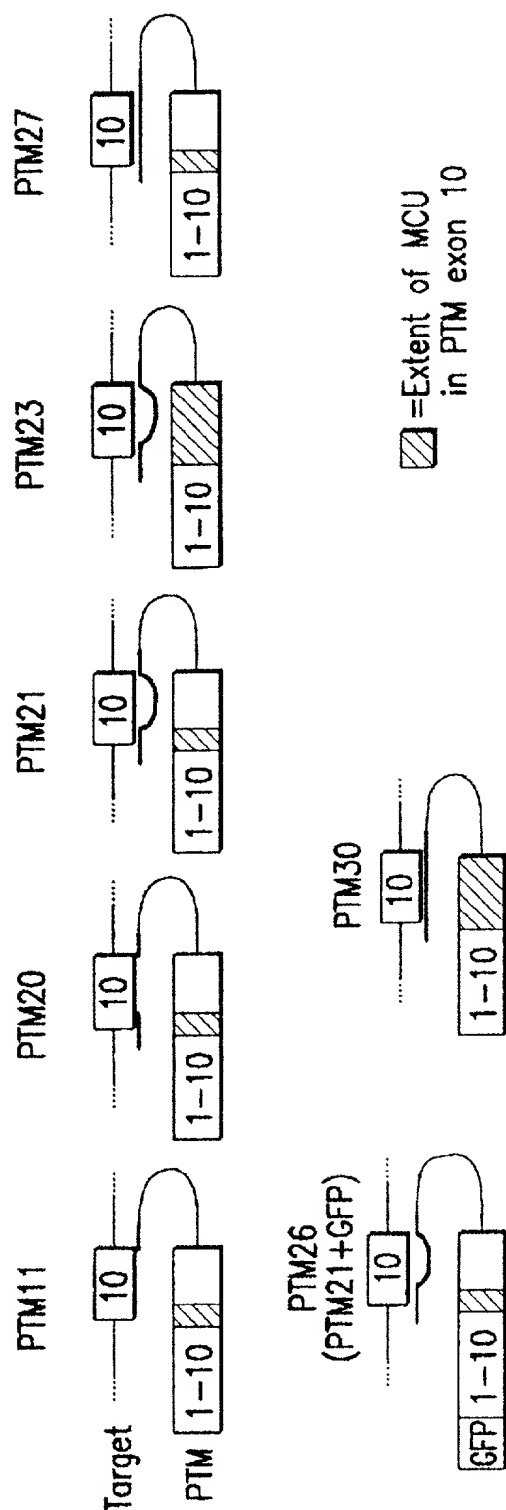


FIG.34C



MCU in exon 10 of PTM
 88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGGTCATGATCATGGCCAGTTAGAACCAAGTGAAGCGAAGATCAAACATTCCCG
 GCCGCATCAGCTTTTCAGGCCAATTCAGTTGGATCATGCCCGGTACCAATCAAGGAGAACATAT
 CTTCGGCGTCAGTTACGACGAGTACCGCTATCCGTCGGTGTATTAAGGCCGTGTCAGTTGGAGGAG

FIG.35

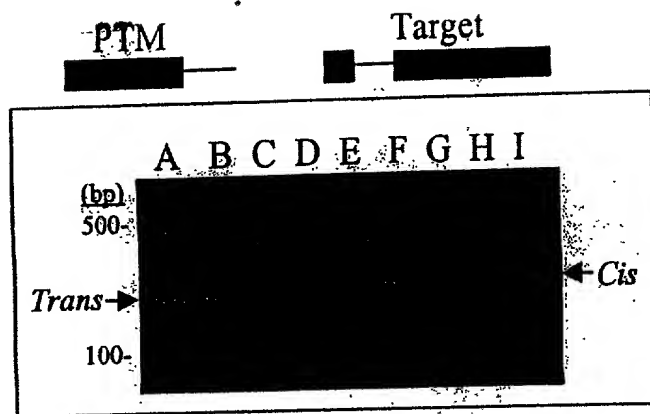


FIG. 36 A

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Cis-spliced product
[Primers CF1+CF111]

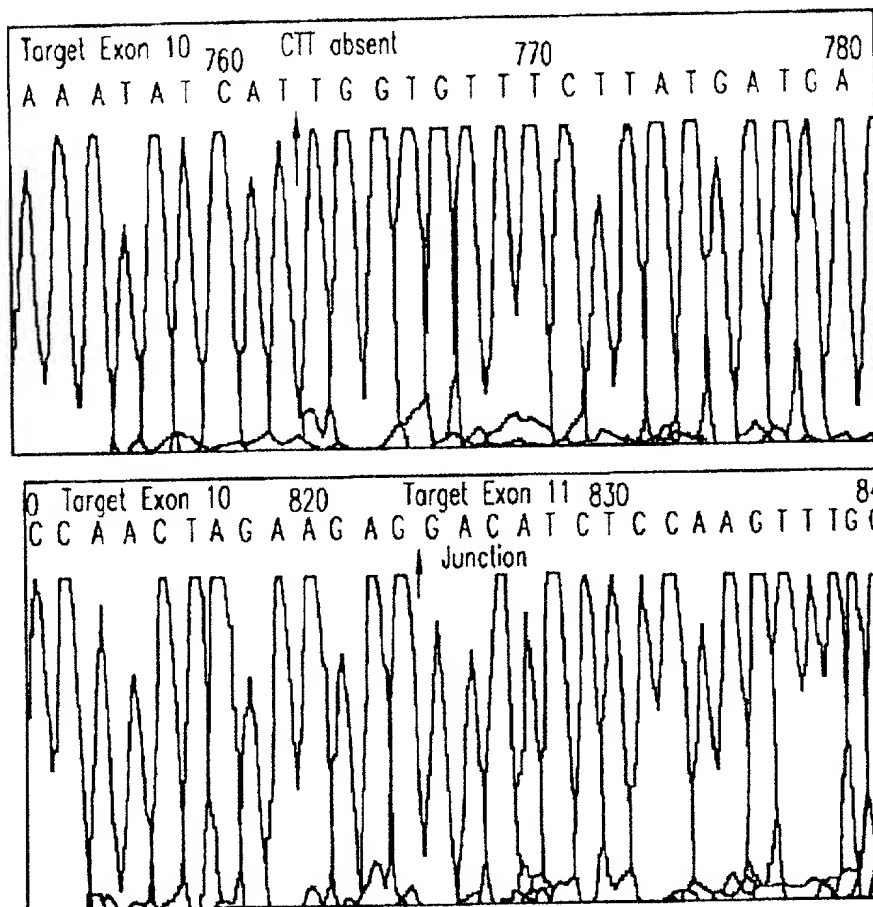


FIG.36A-1

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Trans-spliced product
[Primers CF93+CF111]

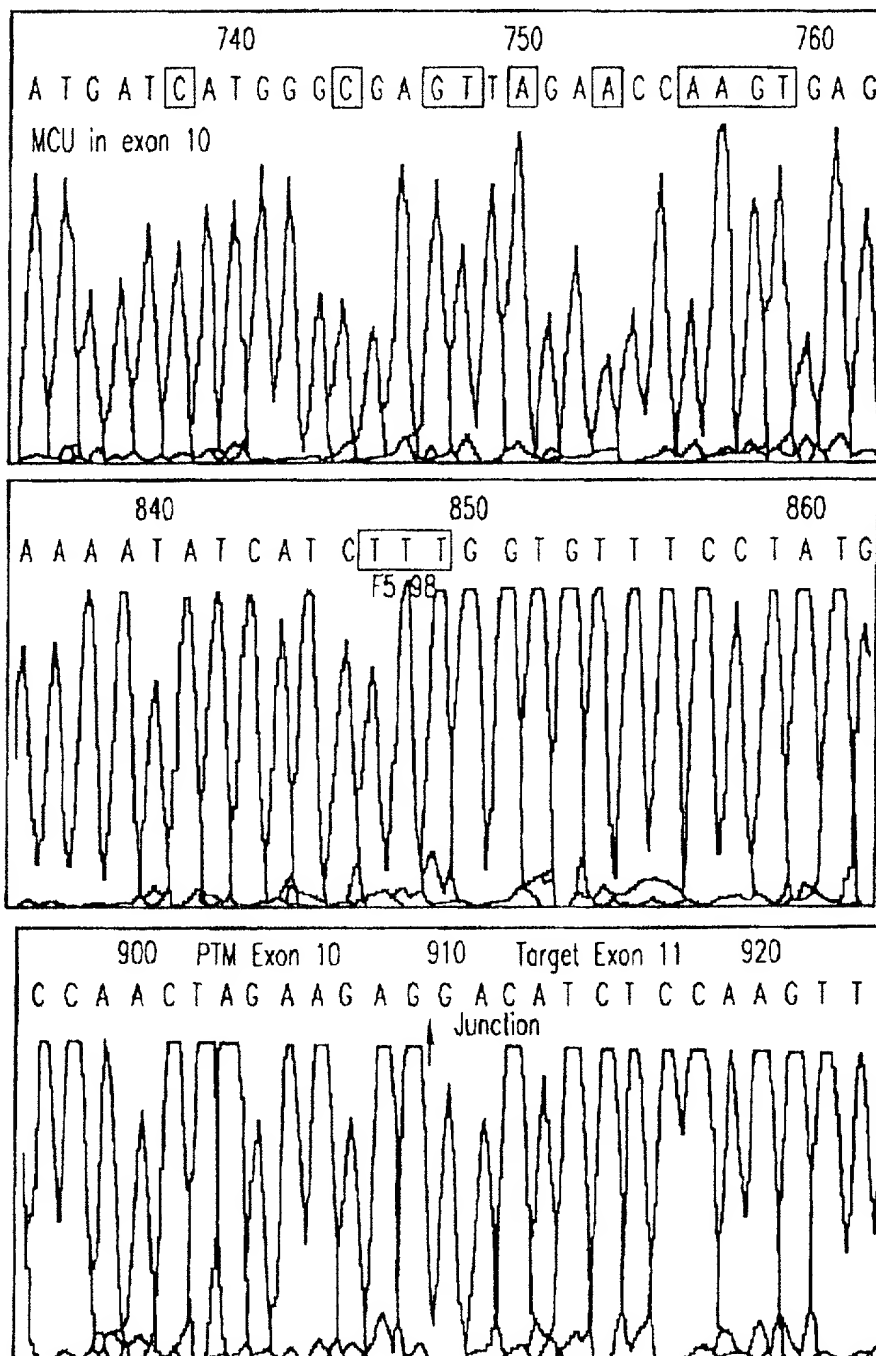


FIG.36B

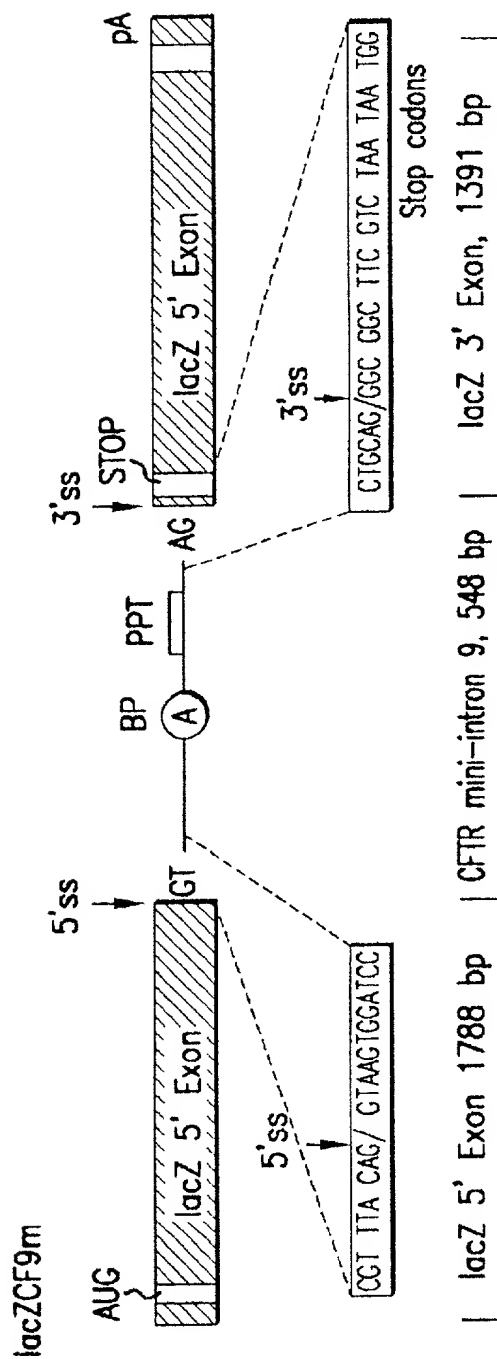


FIG.37A

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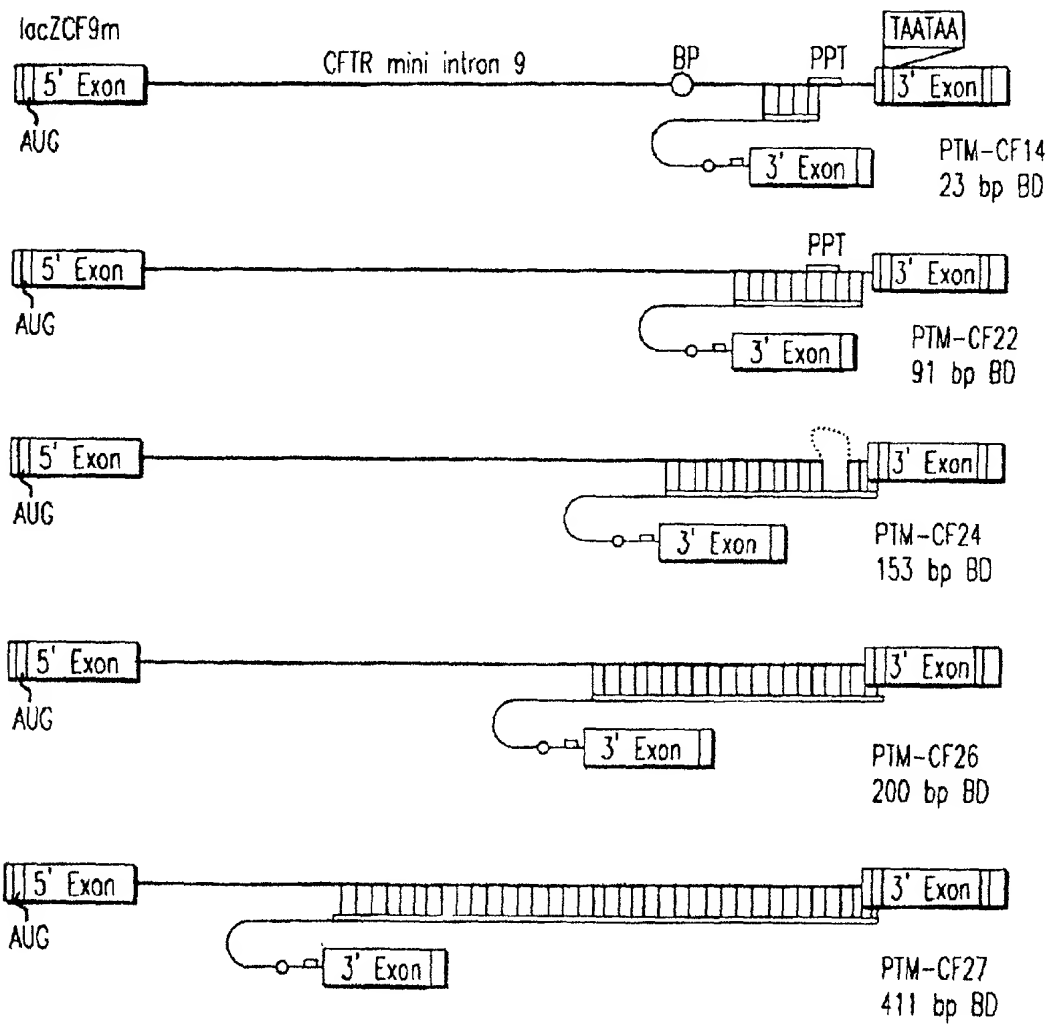
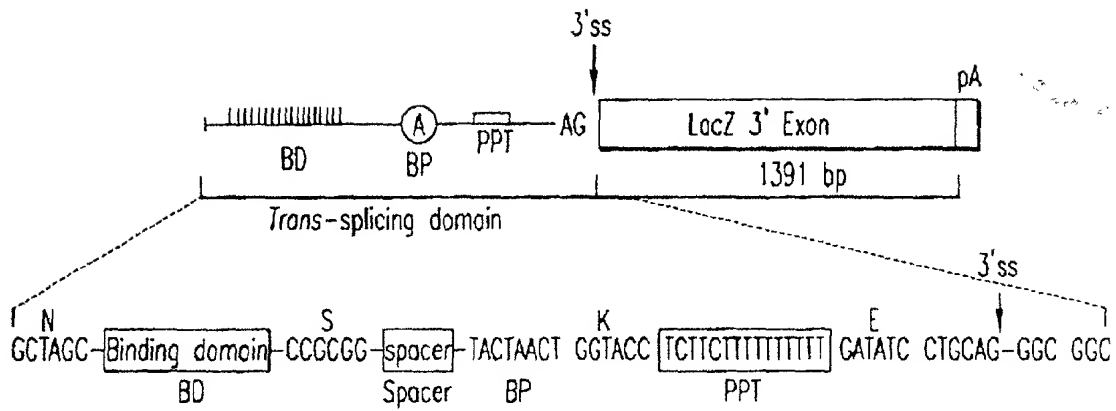


FIG.37B

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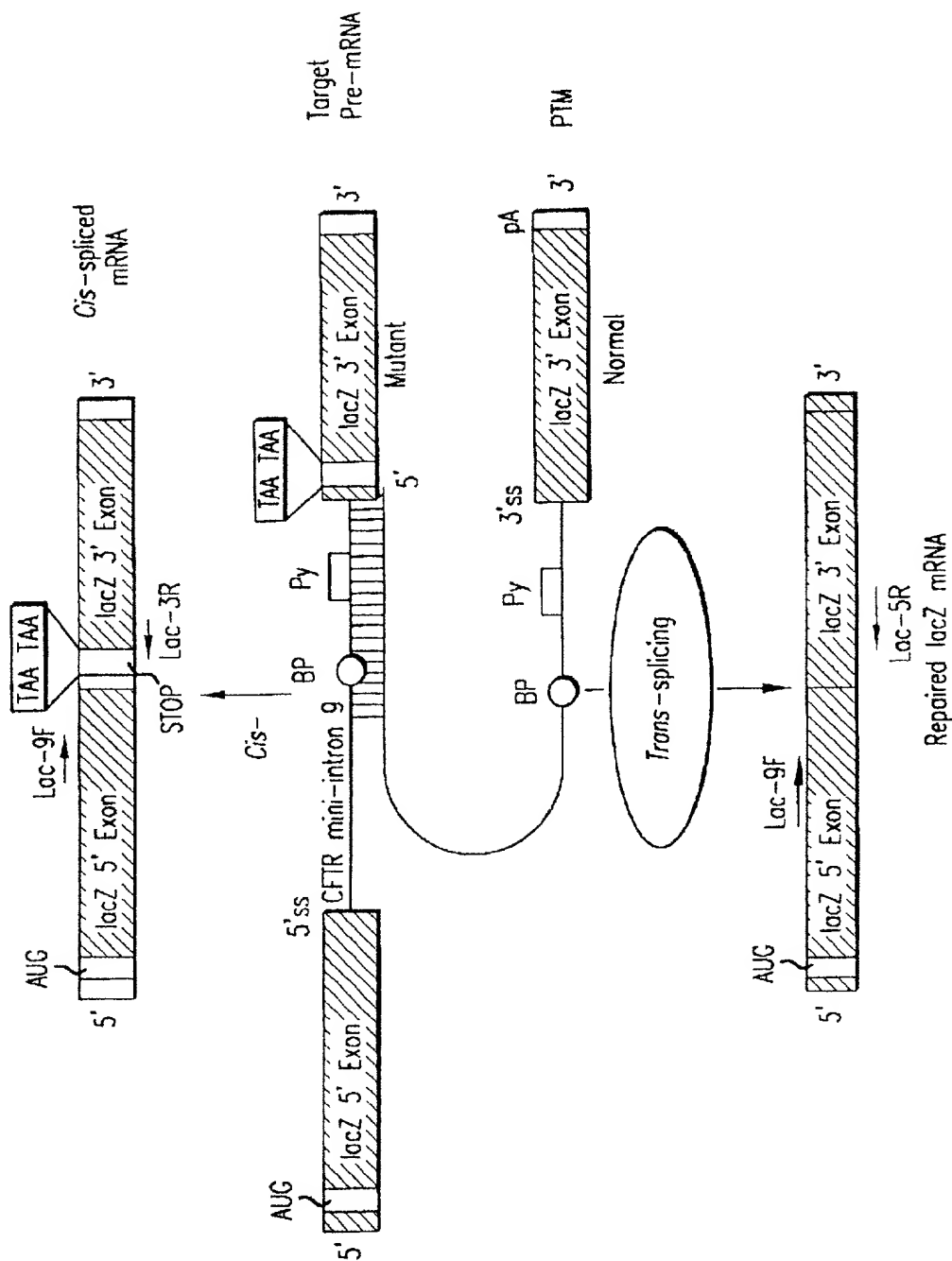


FIG.37C

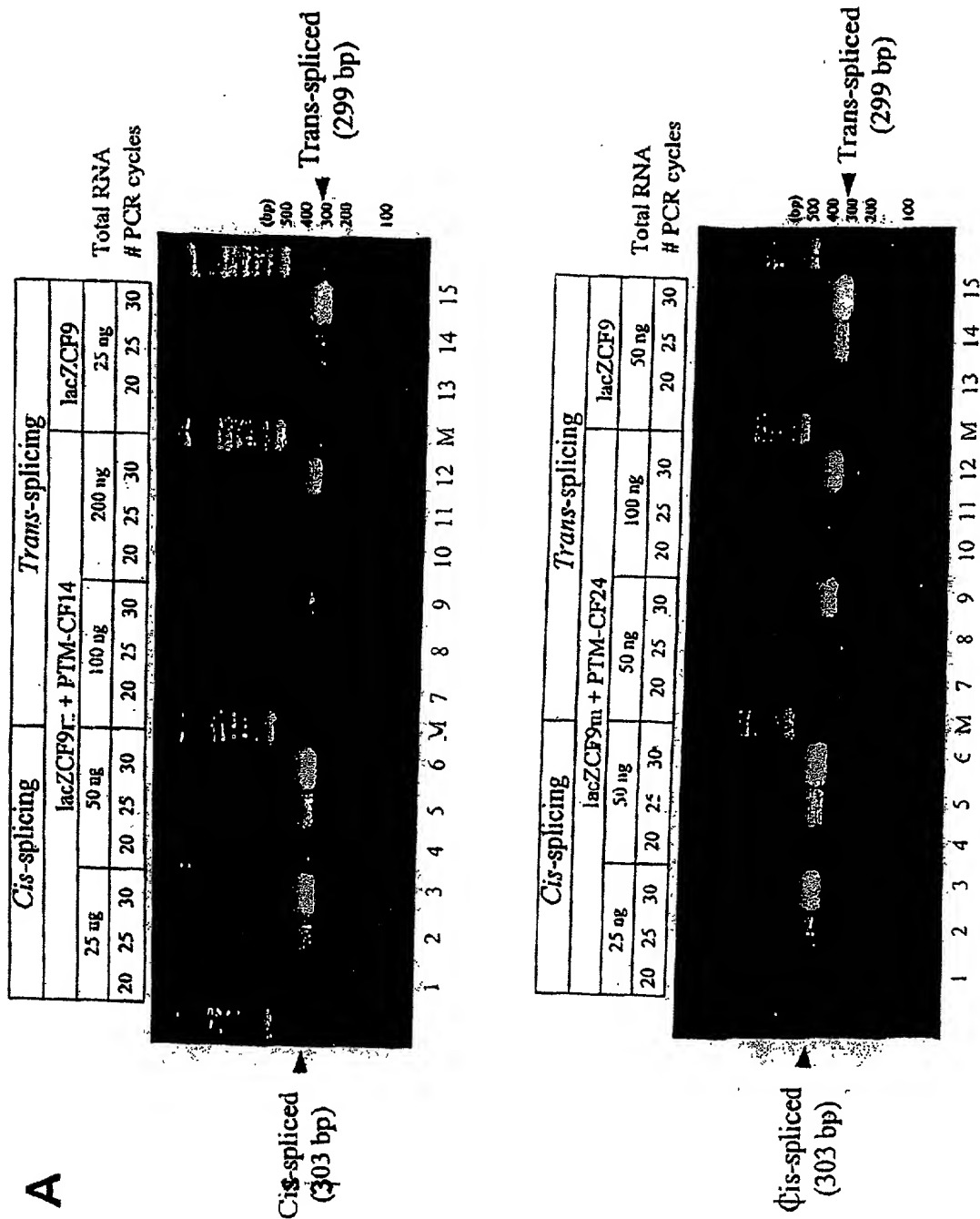


FIG. 38A

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B

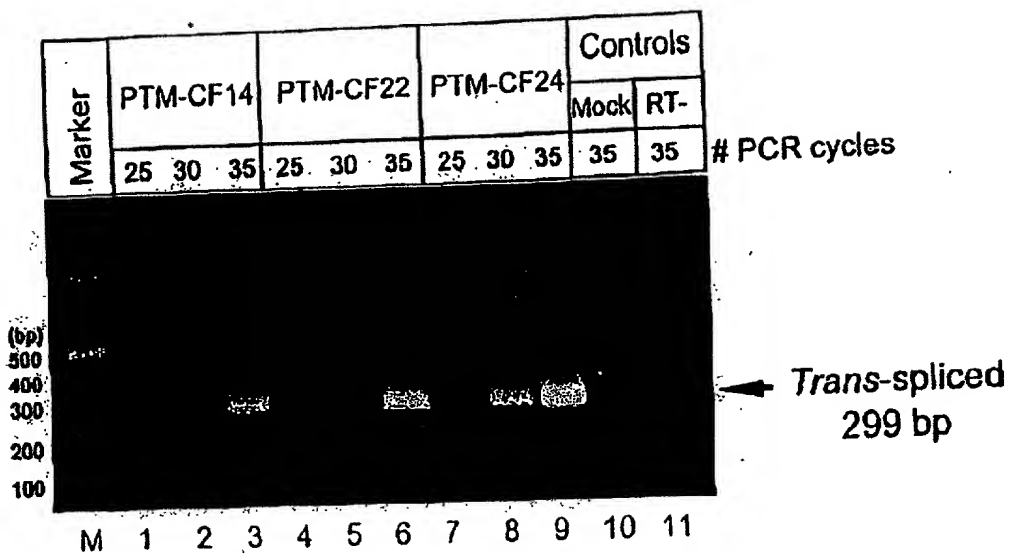


FIG. 38B

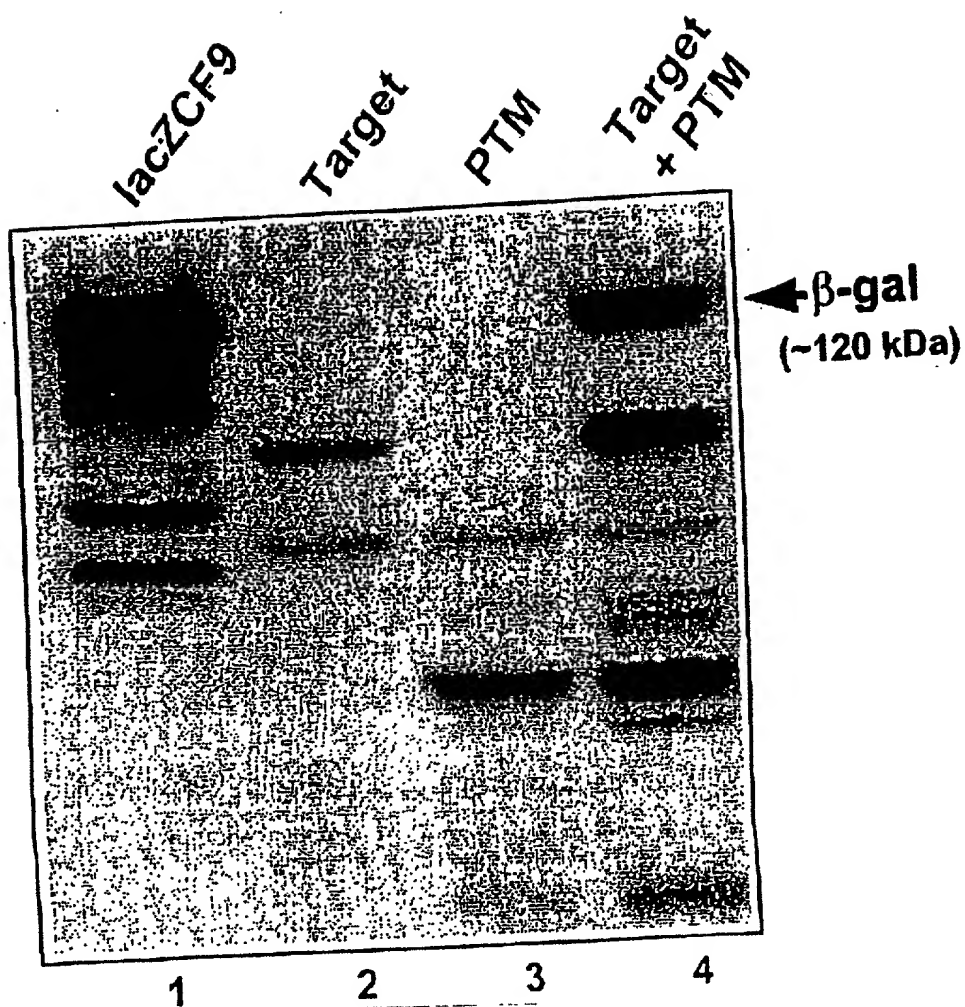


FIG. 39

204040" 2644660

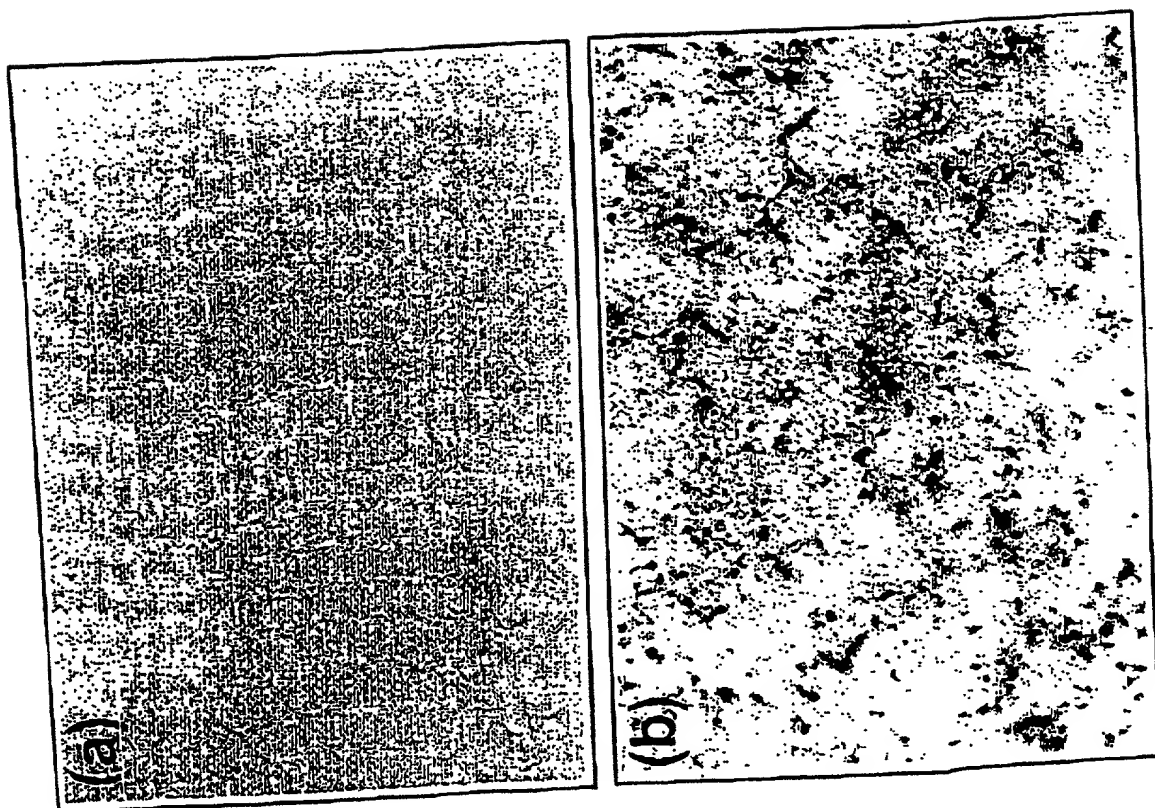


FIG. 40A

A

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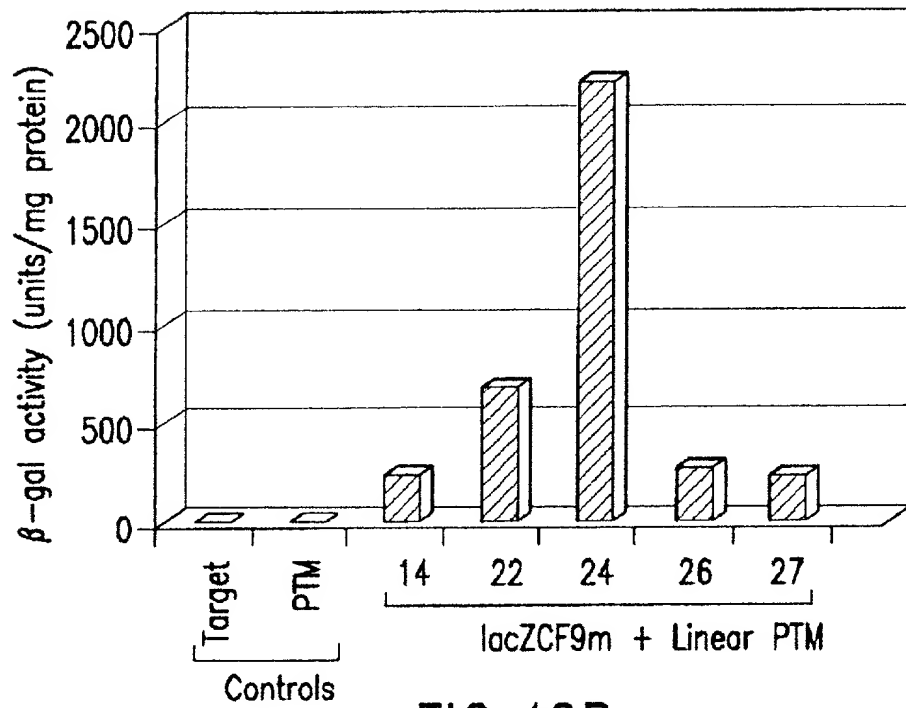


FIG.40B

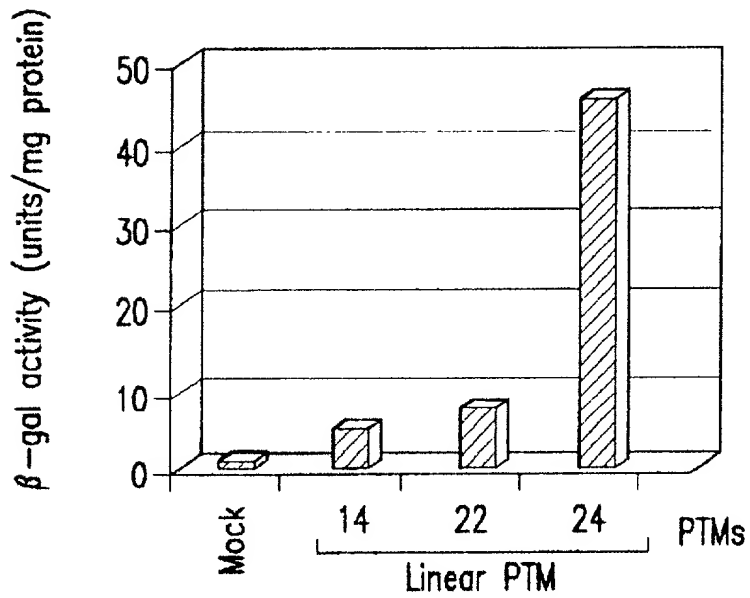


FIG.40C

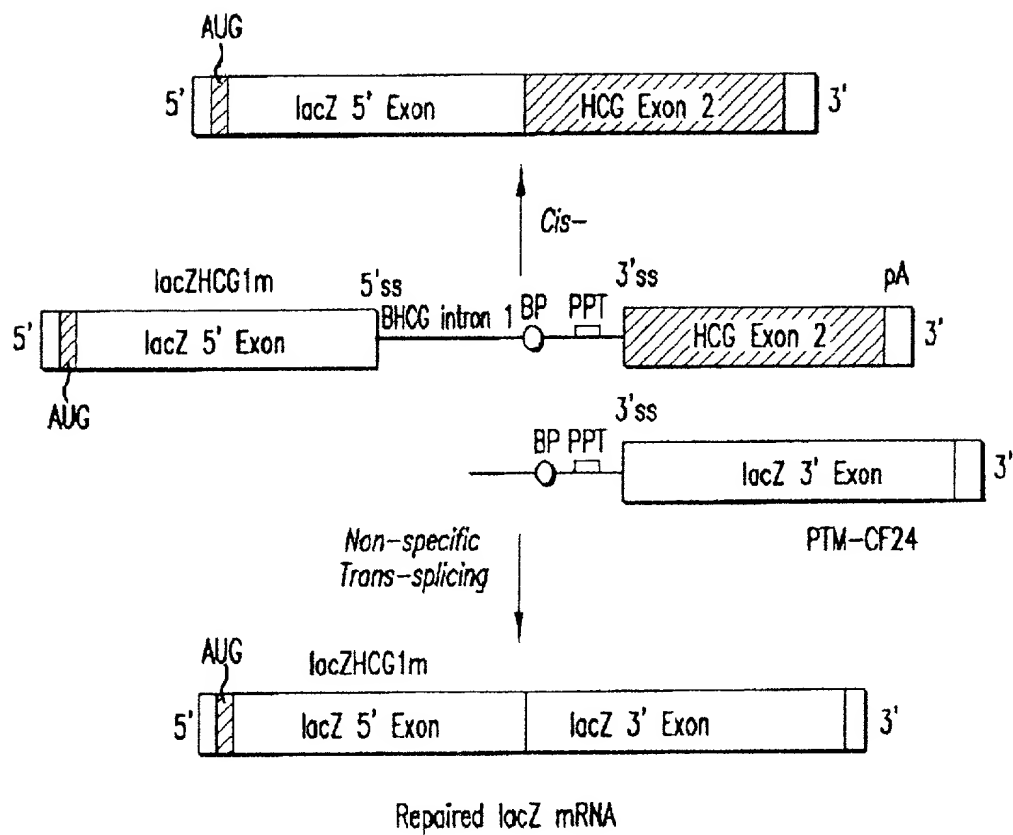


FIG.41A

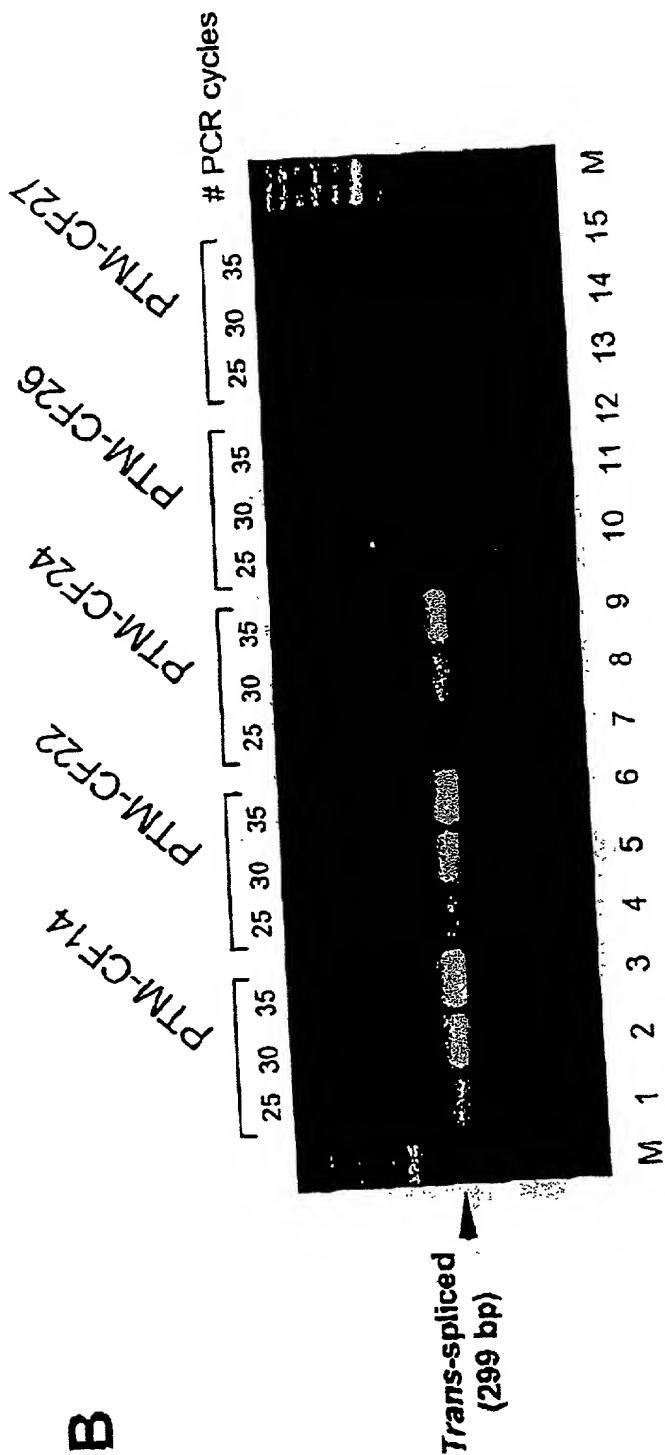


Figure 4B

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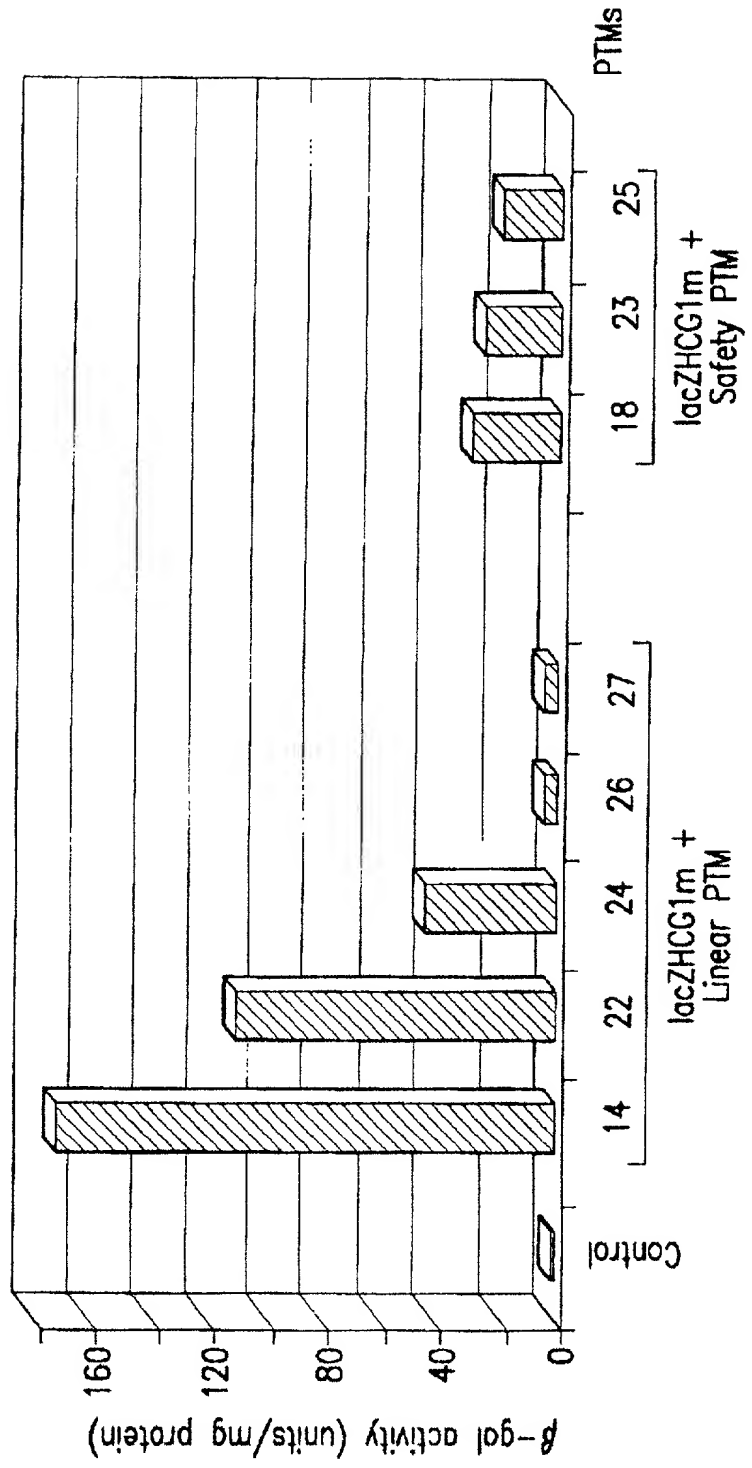


FIG.41C

Exons

1-10

ATGCAGAGGTCGCTCTGGAAAAGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
 GATACAGACAGCGCTTGAATTTGCAGACATATACCAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
 GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTATGCCCTTCGGCGATGTTTTTCTGG
 AGATTTATGTTCTATGGAATCTTTTATATTTAGGGGAAGTCACCAAGCAGTACAGCCTCTCTTACTCGGAAGAATCA
 TAGCTTCCATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCCTCTCTTTAT
 TGTGAGGACACTGCTCTACACCCAGCCATTTTTGGCCTTCATCAGATTGGAATGCAGATGAGAATAGCTATGTTTGT
 TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTGTTAGTCTCCTTT
 CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCAGATTTGCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
 CATGGGGCTAATCTGGGAGTTGTTACAGCGCTCGCCTTCTGTGGACTTGGTTTCTGATAGTCTTGGCCTTTTTTCAG
 GCTGGGCTAGGGAGAATGATGATCAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAGAGCTTGTGATTACCTCAG
 AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA
 AACAGAACCTGAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTCTTT
 GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGAAAAATATCACCACCATCTCATTCT
 GCATTGTTCTGCGCATGGCGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
 CAAAATACAGGATTTCTTACAAAAGCAAGAATAAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG
 AATGTAACAGCCTTCTGGGAGGAGGATTTGGGAATTATTTGAGAAAGCAAAACAAACAATAACAATAGAAAACTT
 CTAATGGTGATGACAGCCTCTTCTTCACTAATTTCTCACTTCTTGGTACTCCTGTCTGAAAGATATTAATTTCAAGAT
 AGAAAGAGGACAGTTGTTGGCGTTCCTGGATCCACTGGAGCAGGCAAGACAGCTTGGCTCATGATGATCATGGGCGAG
TTAGAACAAGTGAAGGCAAGATCAACATTCGGGCGCATCAGCTTTTGCAGCCAATTGAGTTGGATCATGCCGGTA
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCCCTCGGTGATTAAGGCCCTGCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGCTAACCTGATTCGGGCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAAGTGAACACAATGAAATTTCTTCCACTGT
GCTTAATTTTACCCCTCTGAATTTCTCCATTTCTCCATAATCATATTACAACCTGAACCTCGGAAATAAACCCATCATT
ATTAACCTCATTATCAAAATCAGCT

FIG.42

204040" 254F456D

153 bp PTM24 Binding Domain:

Nhe I

153 bp BD underlined

GCTAGC-AATAATGACGAAGCCGCCCTCAGCTCAGGATTCACCTGCCCTCCAATTATCATCCTAAGCAGAGTGATA

TTCTTAATTTGTAAGATTCTATTAACTCATTTGATTCAAAATATTTAAATACTTCCTGTTTCACCTACTCIGCTATGC

Sac II
AC-CCGCGG

FIG.43A

Trans-splicing domain

AATAATGACGAAGCCGCCCTCAGGCTCAGGATTCACITGCCCTCCAATTATCATCCTAAGCAGAAGTGATATCTTA
TTTGTAAGATTCTATTAACCTATTTGATTCAAATATTTAAATACTTCCTGTTTCACTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTCGAATACTAAGTGGTACCTCTTCTTTTTTTTGATATCCTGCAG

Exons 10-24

ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGGAGAATTTCATTCT
 GTTCTCAGTTTTCTGGATTATGCCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTCCTATGATGAATATAGATA
 CAGAAGCGTCATCAAAGCATGCCAAGTAGAAGAGGACATCTCCAAGTTTCAGAGAAAAGACAATATAGTTCTTGGAGAA
 GGTGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTGTATT
 TATTAGACTCTCCTTTGGATACCTAGATGTTTAAACAGAAAAAGAAATTTGAAAGCTGTGTCTGTAAGTATGGC
 TAACAAAAGTAGGATTTGGTCACTTCTAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT
 AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAACTCATGGGATGTGATT
 CTTTCGACCAATTTAGTGCAGAAAGAAATTCATCCTAAGTGAAGCTTACACCGTTTCTCATTAGAAGGAGATGC
 TCCTGTCTCCTGGACAGAAACAAAAAACAATCTTTAAACAGACTGGAGAGTTTGGGAAAAAGCAAGAAATCTATT
 CTCAATCCAATCACTCTATACGAAAATTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT
 CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATTTCTGAGCAGGAGAGGCGATACTGCCCTCGCATCAGCGT
 GATCAGCACTGGCCCCACGCTTCAGGCAAGAGGAGGAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT
 CAGAACATTCACCGAAAGACAACAGCATCCACAGAAAAGTGTCACTGGCCCCCTCAGGCAAACTTGACTGAAGTGGATA
 TATATCAAGAAGGTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT
 TTTTGATGATATGGAGAGCATACCAGCAGTCACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA
 ATTTTGTGCTAATTTGGTGTCTAGTAATTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTCTGTGGCTCCTTGGAA
 ACACTCCTCTTCAAGACAAAGGAATAGTACTCATACTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC
 GTATTATGTGTTTTACATTTACGTGGAGTAGCCGACACTTTGCTTGTATGGGATTCTTCAGAGGTCTACCACTGGTG
 GATACTCTAATCACAGTGTGCAAAATTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCTCA
 ACAGGTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAGATATAGCAATTTGGATGACCTTCTGCCCTCTTACCAT
 ATTTGACTTCATCCAGTTGTTATTAATTTGATTTGGAGCTATAGCAGTTGTCCAGTTTTACAACCTACATCTTTGTT
 GCAACAGTGCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAAACCTCACAGCAACTCAAACAAGTGG
 AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTGTTACAAGCTTAAAGGACTATGGACACTTCGTGCCCTTCGAGC
 GCAGCCTTACTTTGAAACTCTGTTCCACAAAGCTCTGAATTTACATACTGCCAACTGGTCTTGTACCTGTCAACACTG
 CGCTGGTTCCAAATGAGAATAGAAATGATTTTGTATCTTCTTCATTGCTGTTACCTTCATTTTCAACAACAG
 GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGCAGTGGCTGTAAACTC
 CAGCATAGATGTGGATAGCTTGATGGATCTGTGAGCCGAGTCTTTAAGTTCAATTGACATGCCAACAGAAGGTAAACCT
 ACCAAGTCAACCAAAACCATACAAGATGGCCAAGTCTCGAAAGTTATGATTATTGAGAATTCACACGTGAAGAAAGATG
 ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA
 GAACATTTCTTCTCAATAAGTCTCGCCAGAGGCTGGGCCCTCTGGGAAGAACTGGATCAGGAAGAGTACTTTGTTA
 TCAGCTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGCTTGGGATTCATAACTTTGCAAC
 AGTGGAGGAAACCTTTGGAGTGATACCACAGAAAGTATTTATTTTTCTGGAACATTTAGAAAAAAGTTGGATCCCTA
 TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCAGATGAGGTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG
 AAGCTTGACTTTGTCTTGTGGATGGGGCTGTGCTTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
 TTCTCAGTAAGCGGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAATAATTAGAAG
 AACTCTAAAACAAGCATTTGCTGATTGCACAGTAATTCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
 TTTTGGTCTATAGAAGAGAACAAAGTGGGCAGTACGATTCATCCAGAAAGTGTGAACGAGAGGAGCCTCTTCCGGC
 AAGCCATCAGCCCTCCGACAGGTGAAGCTTTTCCCACCGGAAGTCAAGCAAGTGAAGTCTAAGCCCCAGATTGC

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATCATTAG

FIG.43B

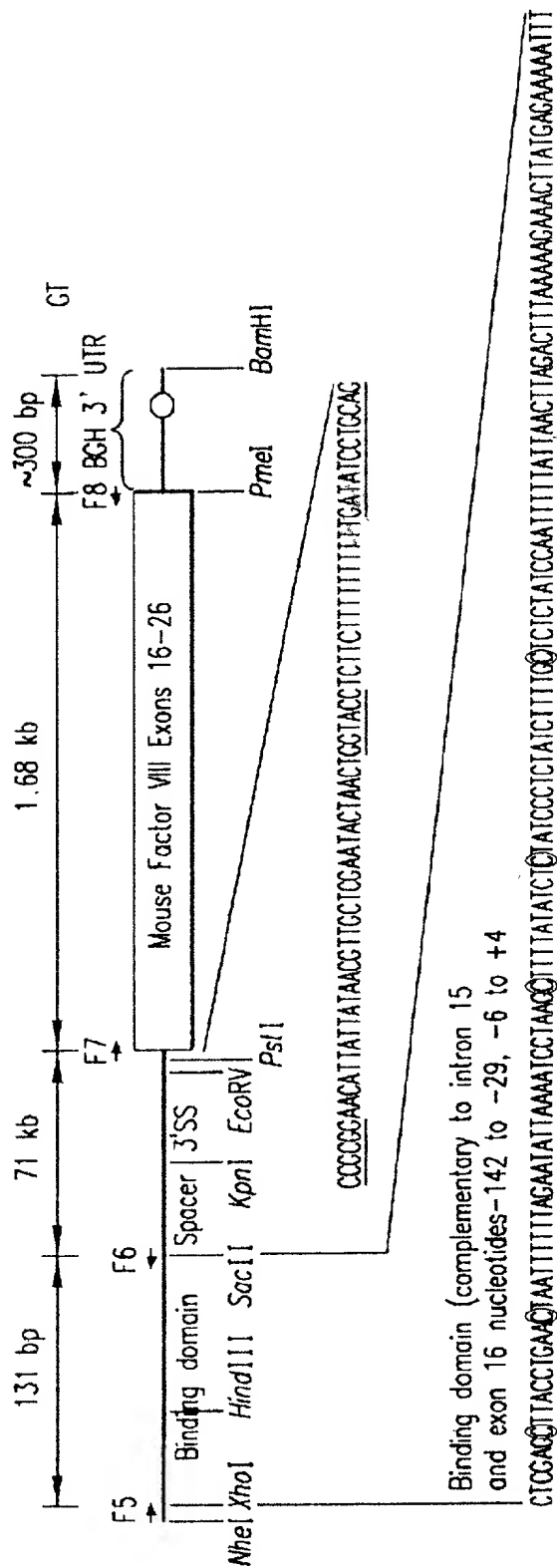


FIG. 44A

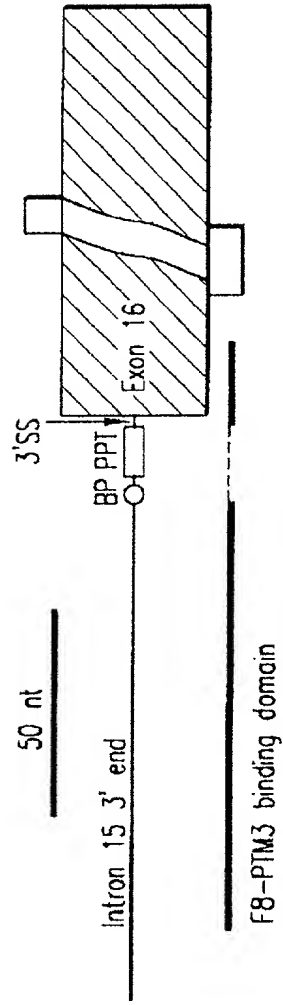


FIG.44B

[illegible]

Chicken β -actin
Promoter

Nucleotide changes are shown in blue

Boxed=Cat box, TATA box

Boxed+Arrow=Transcription Start

Oval=Downstream elements

Bold=Binding domain

Italicized=Spacer+PPT+BP+AC dinucleotide

Sequence not included in construct

CGCGCCCTCGCCGGCGCCCGCGGCTCTACTGACCGGTTACTCCACAGGTGAG
CGGGCGGACGGCCCTCTCGTCGGGCTGTAATTAGCGCTCGTTTAAACAGGCT
TGTTCTTTTCTGTCGTCGGTGAAGGCTGACGGGCTCCGGAGCAATTGGTA

Extent of promoter in above construct

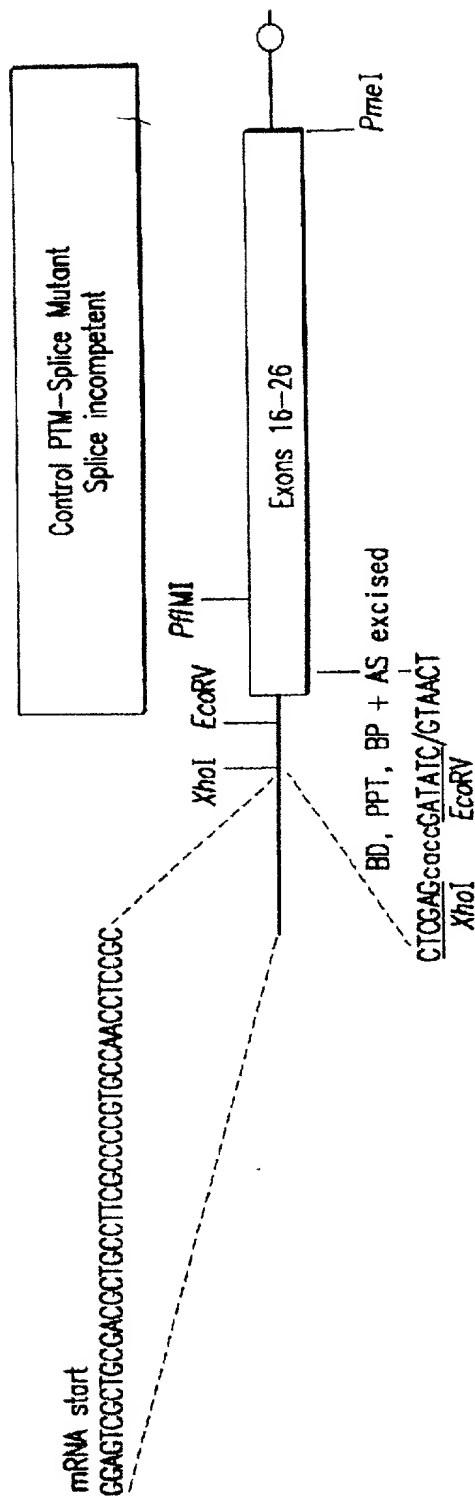
Extent of promoter in original construct

CMV enhancer
CBA promoter
Exon 1 Intron 1 (partial)

$$\begin{aligned} F13+F2 &= 235+106=341 \text{ bp} \\ F13+F4 &= 235+315=550 \text{ bp} \end{aligned}$$

Chicken Beta Actin Promoter (including exon 1 and part of intron 1)

FIG. 44C



Method:
Excise TSD and part of exon 16 with
XhoI and PflMI and ligate in a PCR product that:
1) eliminates the TSD and splice acceptor site
2) inserts EcoRV adjacent to exon 16
3) restores the coding for exon 16

FIG.45

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Repair of Factor VIII
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice
after IV PTM-FVII intraportal infusion
(100 μ gDNA)(n=3)

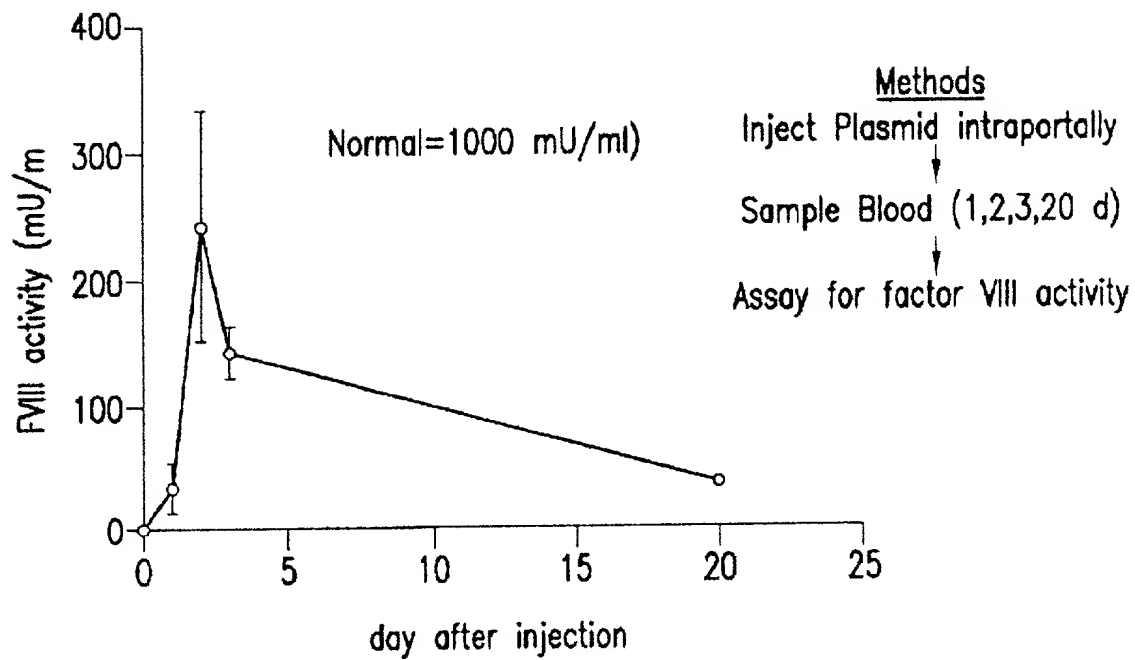


FIG.46

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

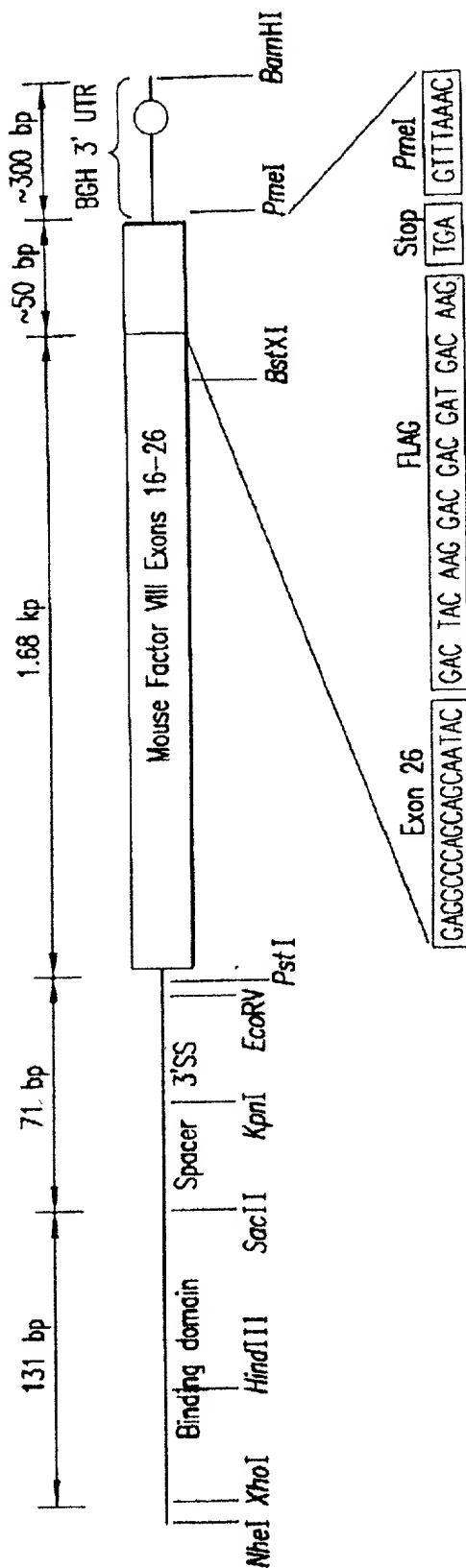
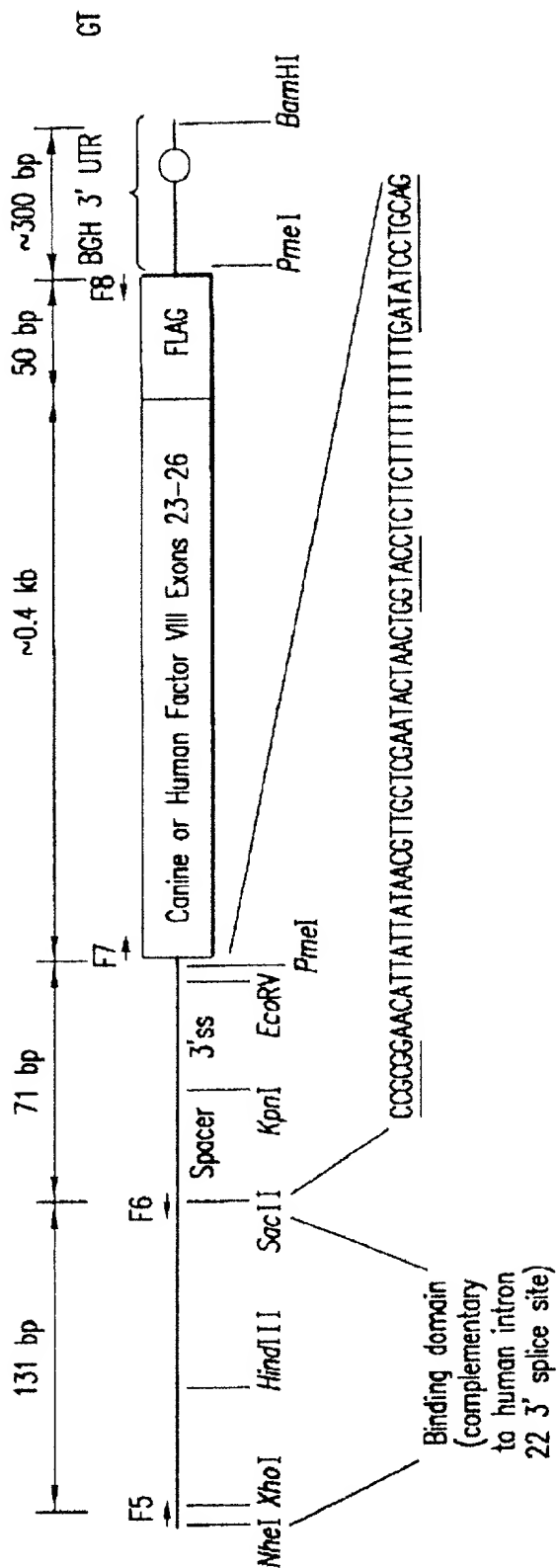


FIG.47A

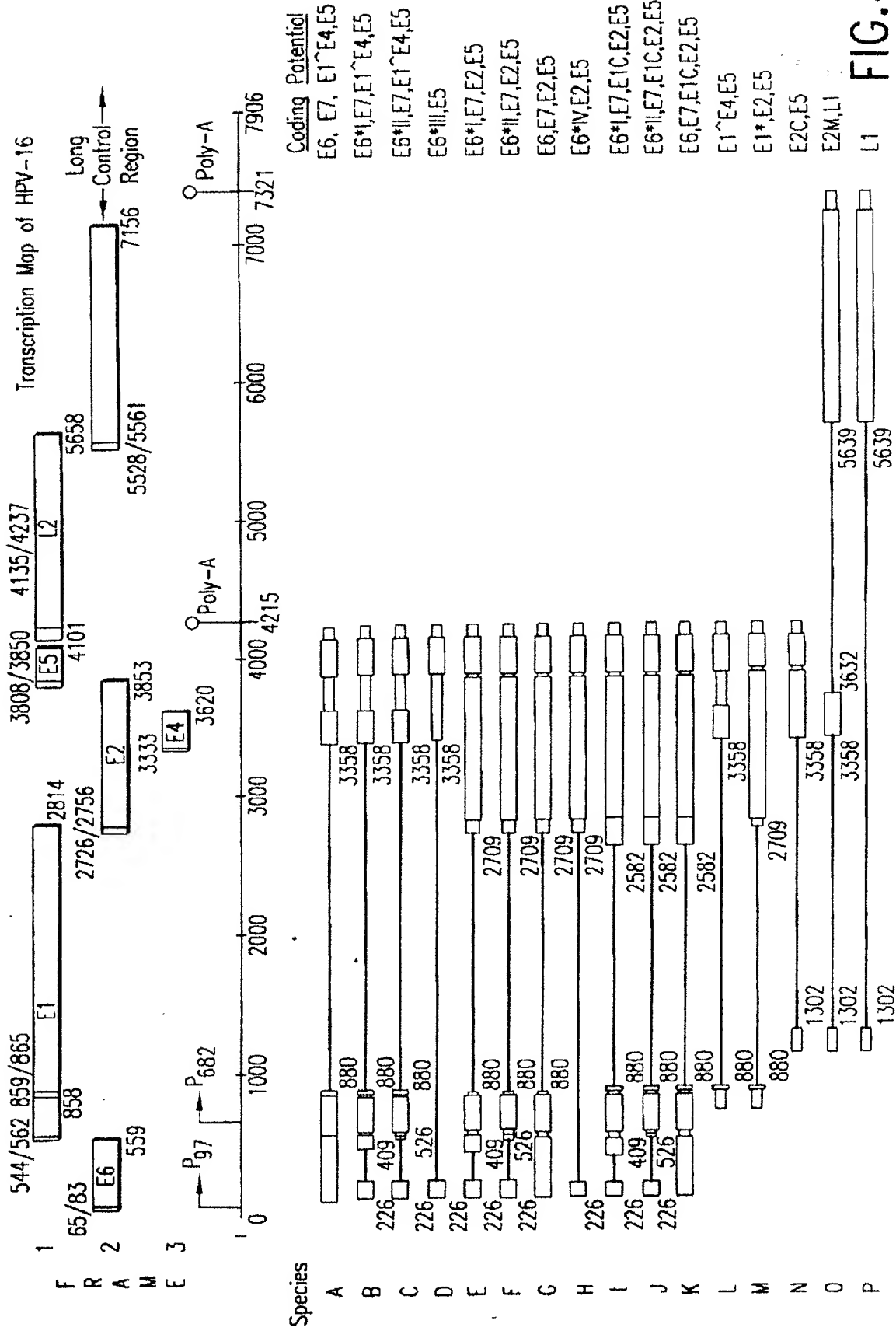
71 2 91



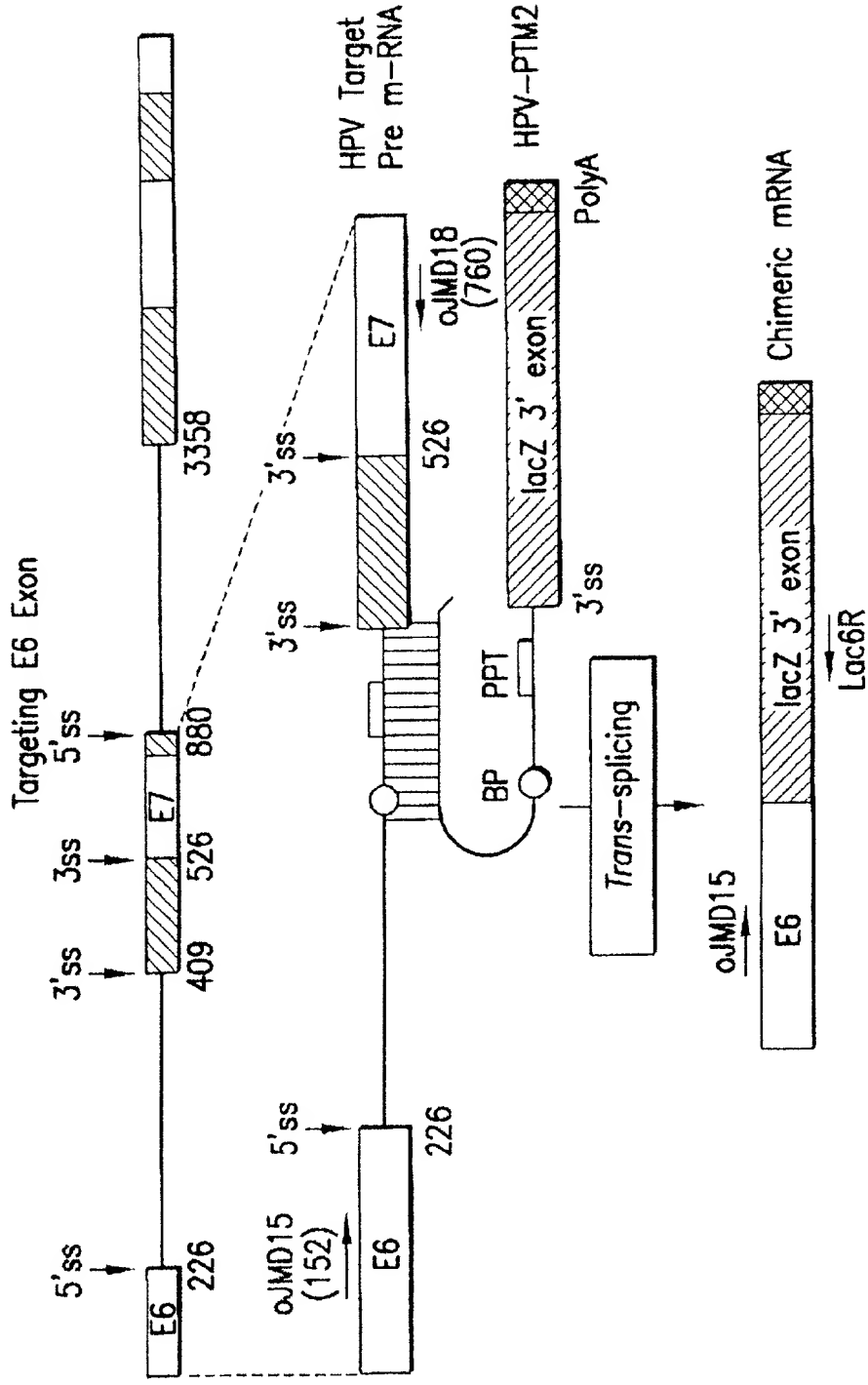
FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG.47B

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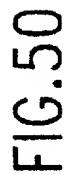


SMART Strategy to Disrupt the Expression of Human Papillomavirus Type 16



SMART Strategy by 3' Exon Replacement: Schematic diagram of HPV-PTM2 binding to the 3' splice site of the HPV type 16 target pre-mRNA

FIG.49



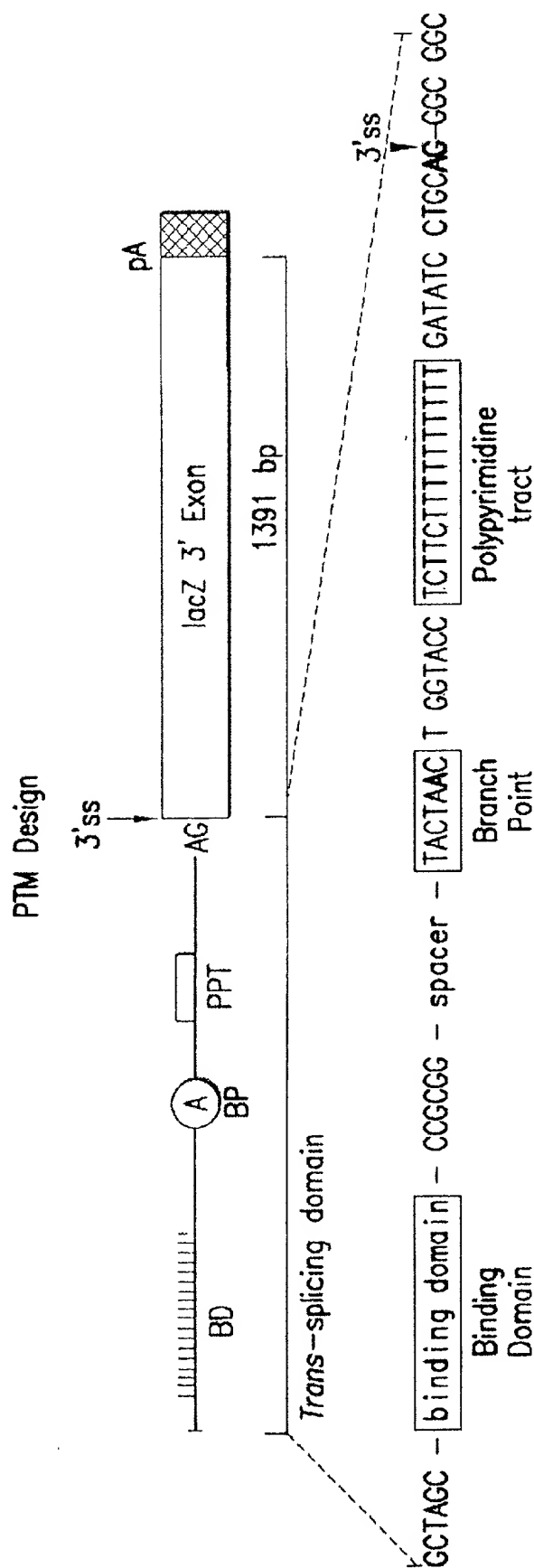


FIG.51

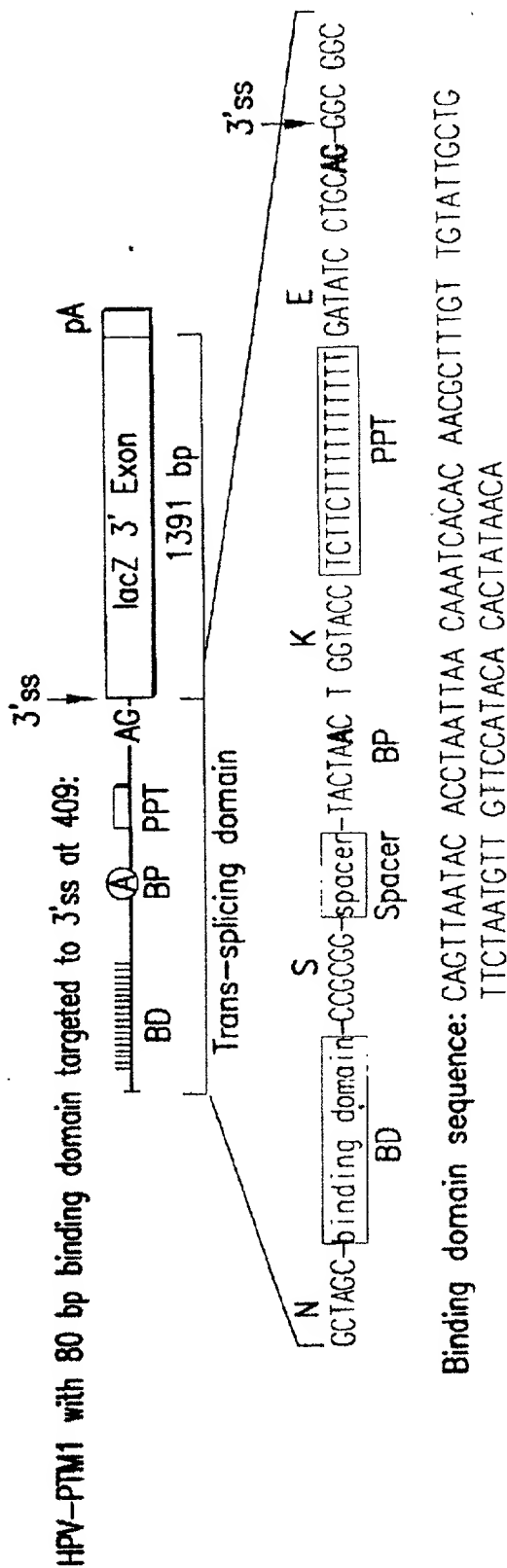
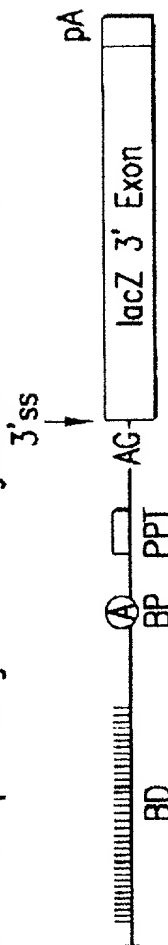


FIG.52A

HPV-PTM2 with 149 bp binding domain targeted to 3'ss at 409:

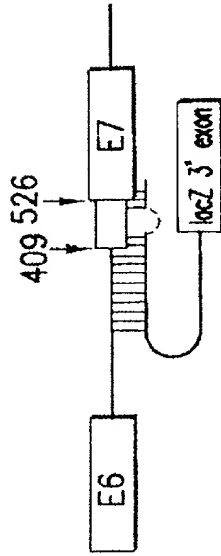


Binding domain sequence: CAGTTAATAC ACCTAATTAA CAAATCACAC AACGCTTTGT TGTATTCGIC TTCTAATGTT GTTCCATACA CACTATAACA ATAAATGCTA TACTACACTAA TTTTAGAATA AAACITTTAA CATTATATCAC ATACAGCATA TCGATTCCC

FIG.52B

Binding Domains of HPV-PTM3 and 4

HPV-PTM3 Binding domain (covers both 3'ss at 409 and 526; has 53 bp bubble)
 GATGATCTGCAACAAGACATACATCGACGGTCCA(53 nt bubble)CTTCAGGACACAGTGGCTTTTGAC
 AGTTAATACACCIAATTAAACAATCACACAACGGTTTGTTGTTATTCAGTTCTAATGTTGTTCCATACACACTA
 TAACAAT



HPV-PTM4 Binding domain (covers both 3'ss at 409 and 526; has 76 bp bubble)
 GATGATCTGCAACAAGAC(76 nt bubble)GACACAGTGGCTTTTGACAGTTAATACACCCTAATTAAACAAATC
 ACACAACGGTTTGTTGTTATTCAGTTCTAATGTTGTTCCATACACACTATAACAAT

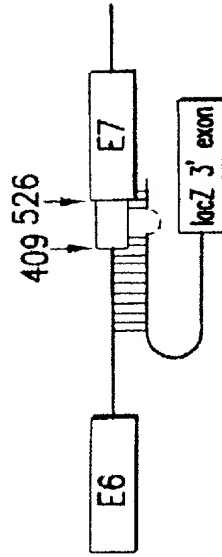
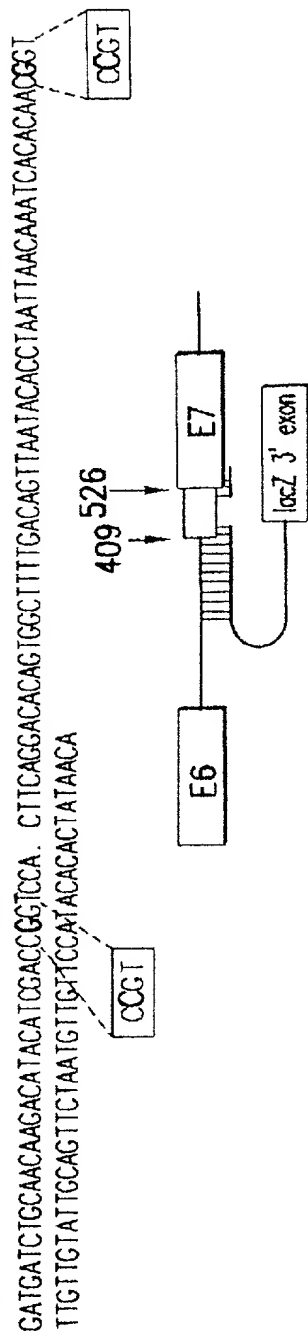


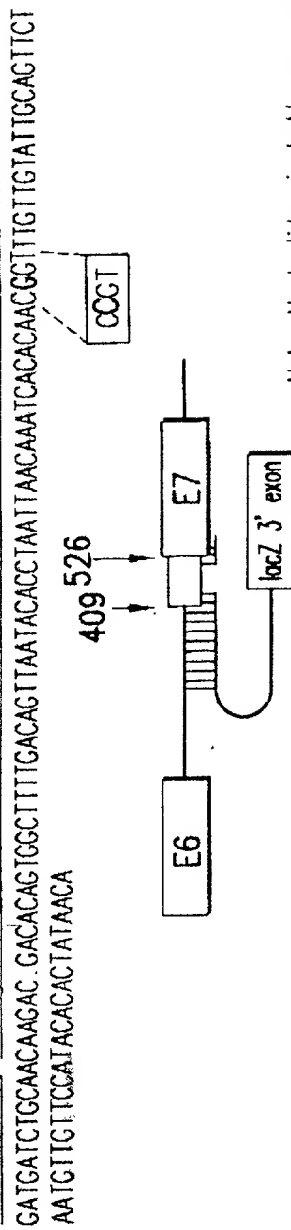
FIG.53

HPV-PTM5 and 6

HPV-PTM5, Binding domain (140 nt, has **53 nt bubble**, covers 3'ss at position 409 and 526)



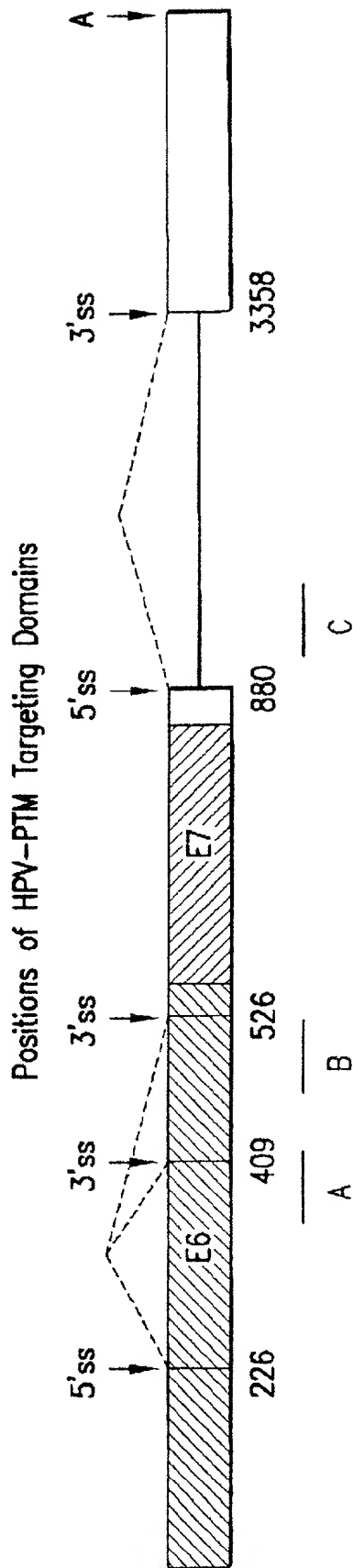
HPV-PTM6, Binding domain (117 nt, has **76 nt bubble**, covers 3'ss at position 409 and 526)



Note: Nucleotides in bold are modified to prevent PTMs cryptic splicing

FIG.54

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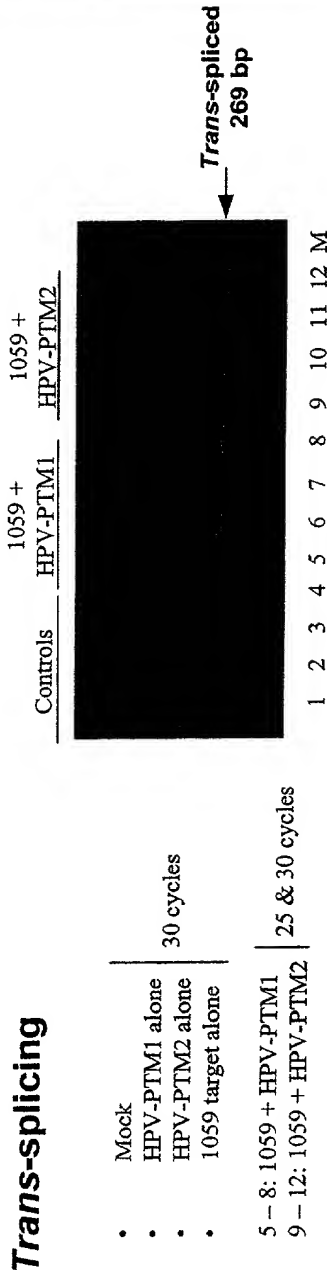
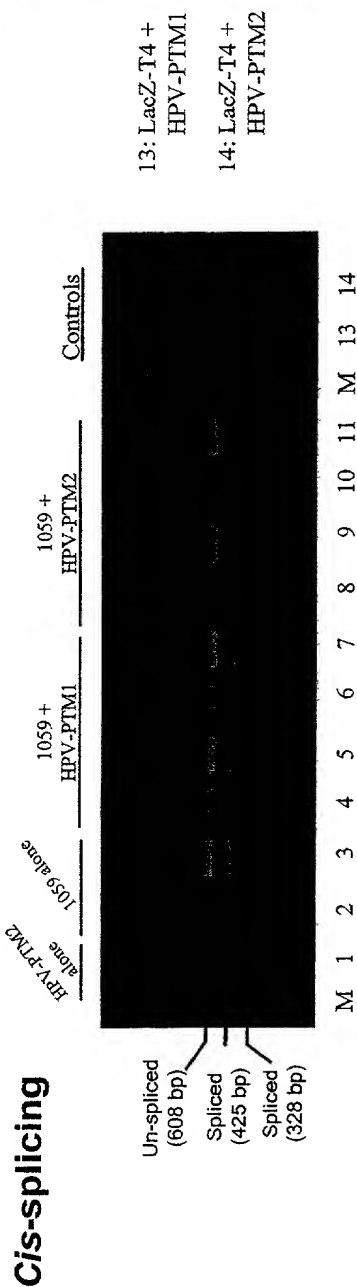


PTM	Binding Domain	
	Region	Size (nt)
HPV-PTM1	A	80
HPV-PTM2	A	149
HPV-PTM5	A+B	140
HPV-PTM6	A+B	117
HPV-PTM8	C	104
HPV-PTM9	C	174

FIG.55

80 2 91

Trans-splicing Efficiency of HPV-PTMs in 293T Cells



RT-PCR Analysis of total RNA

INTRON

FIG. 56

Trans-splicing between target pre-mRNA and
PTM is accurate (293T cells)

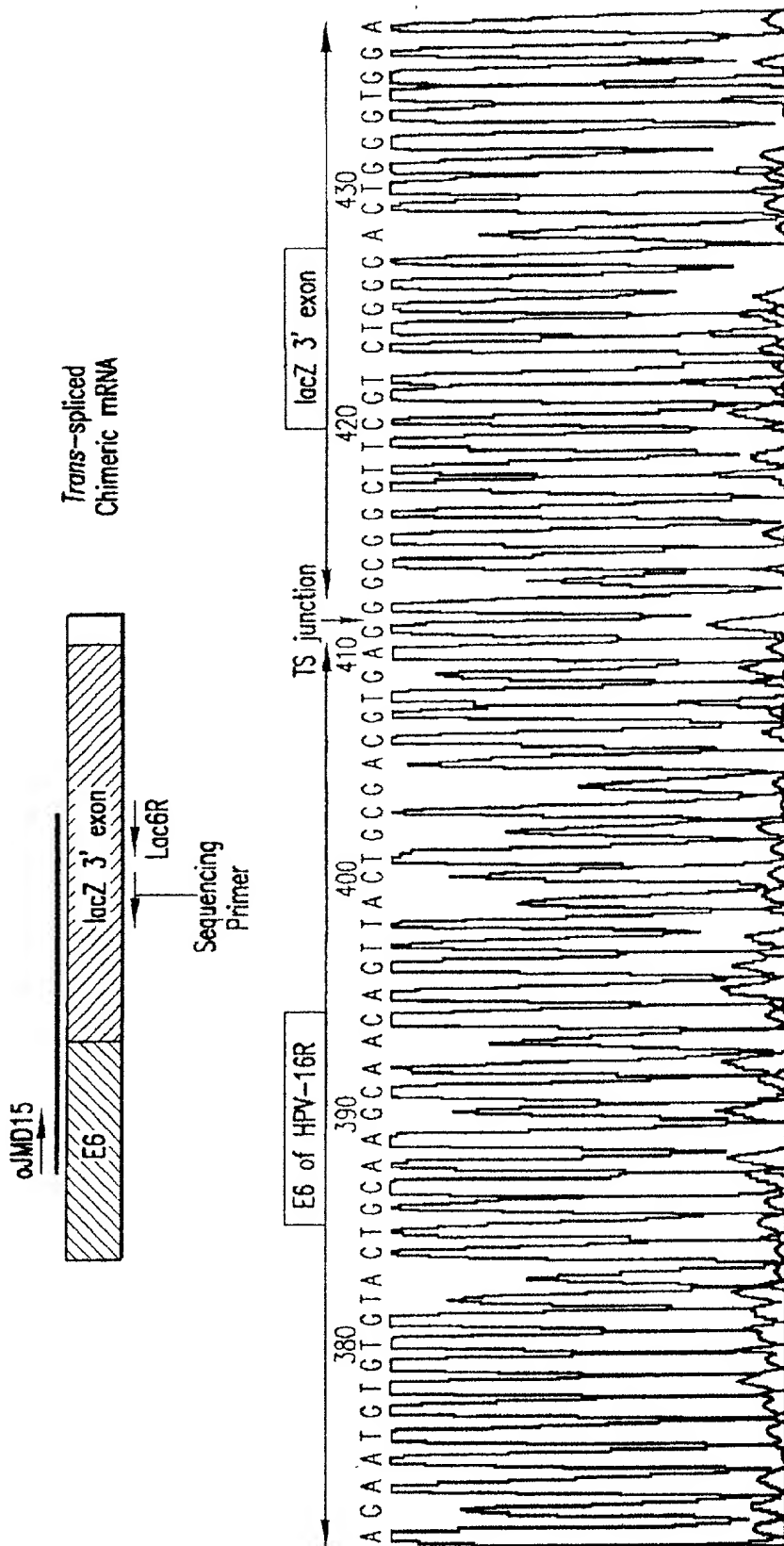
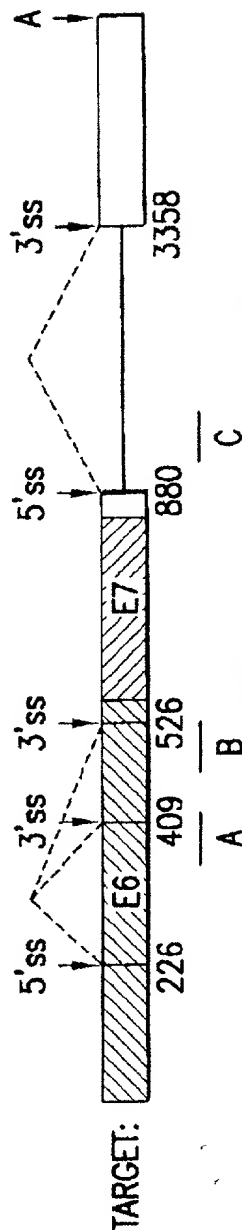


FIG.57

Trans-splicing in 293 Cells (Co-transfections)



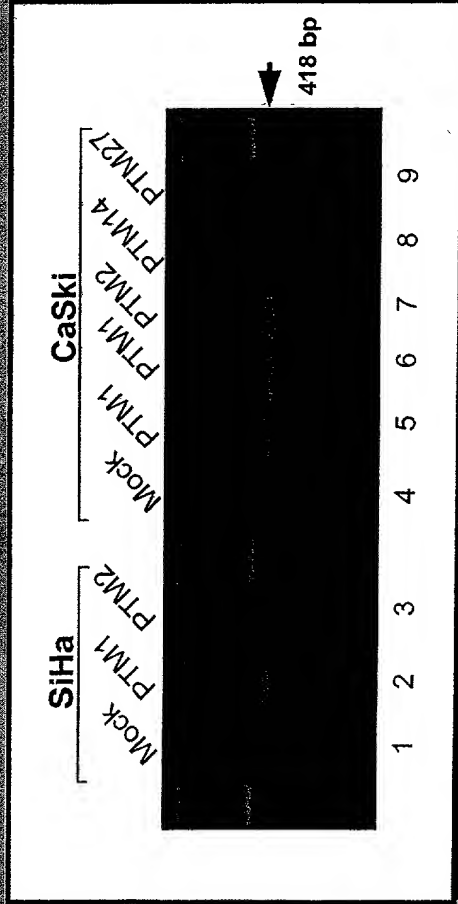
PTM	Binding Domain		% trans-spliced	
	Region	Size (nt)	226 sd	880 sd
HPV-PTM1	A	80	69	0.6
HPV-PTM2	A	149	45	0.9
HPV-PTM5	A+B	140	55	0.8
HPV-PTM5ΔBP/PPT	A+B	140	0.5	0.2
HPV-PTM6	A+B	117	59	1
HPV-PTM8	C	104	7	37
HPV-PTM9	C	174	14	22
CF-PTM27	CF intron	411	0	0

Quantification of Trans-splicing efficiency using real-time QRT-PCR

FIG.58

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Trans-splicing into Endogenous HPV Pre-mRNA Target in SiHa & CaSki Cells



SiHa: Single copy
CaSki: ~400-500 copy/11

Trans-spliced
Chimeric mRNA

RT-PCR Analysis of total RNA

RT-PCR Conditions

- Total RNA: 400 ng/rxn
- Primer's: oJMD15 + Lac16R
- # Cycles : 35
- Expected product : 418 bp

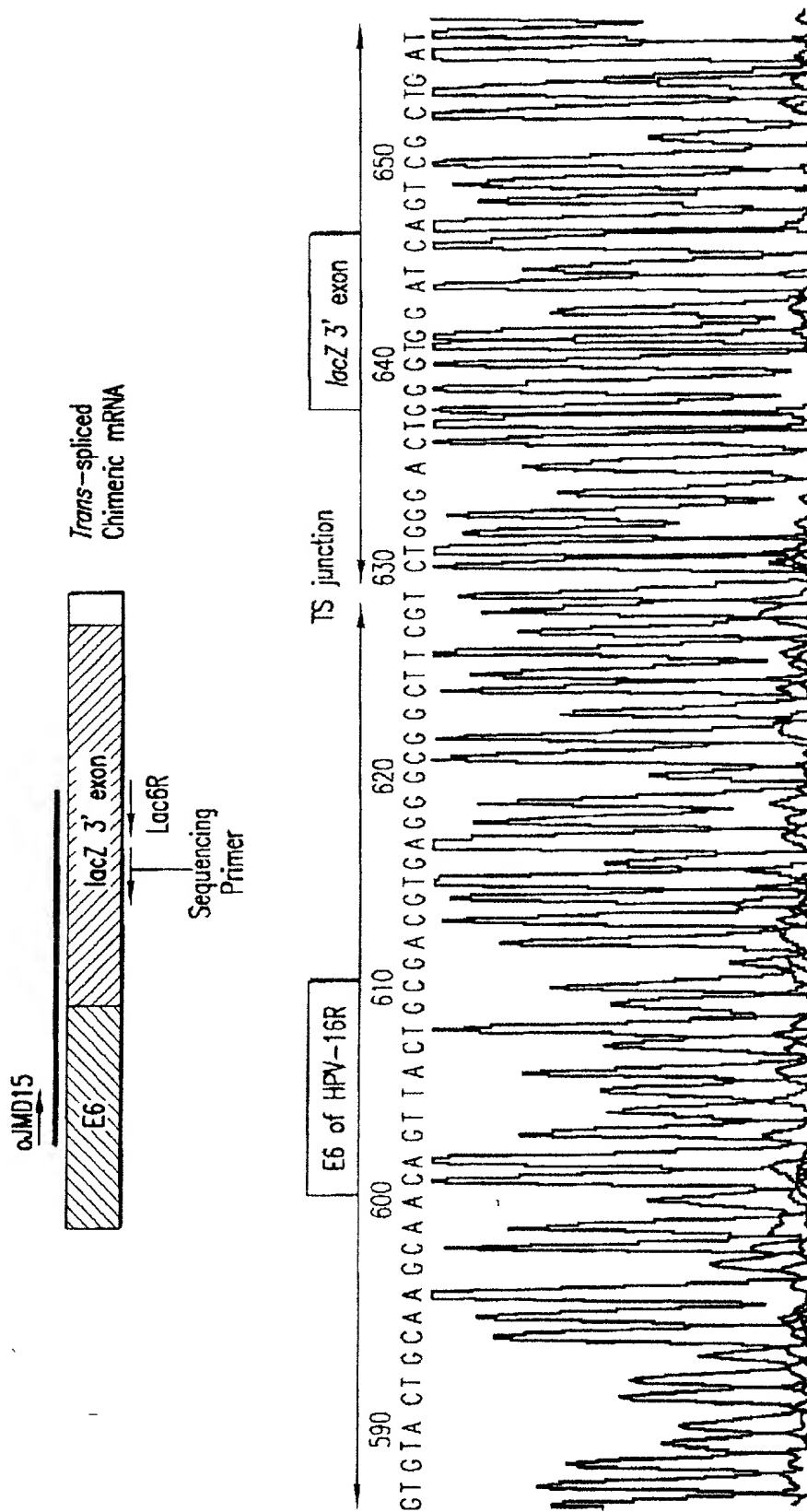
Details

- PTM1, PTM2 : HPV targeted, specific
- PTM14 : CF targeted, non-specific, has 23 bp BD
- PTM14 : CF targeted, non-specific, has 411 bp BD

INTRONN

FIG. 59

Accurate Trans-splicing of HPV-PTM1 in Si Ha Cells (Endogenous target pre-mRNA)



Quantification of trans-splicing efficiency using real-time QRT-PCR

FIG.60

Trans-splicing in SiHa Transfections
(Endogenous target)

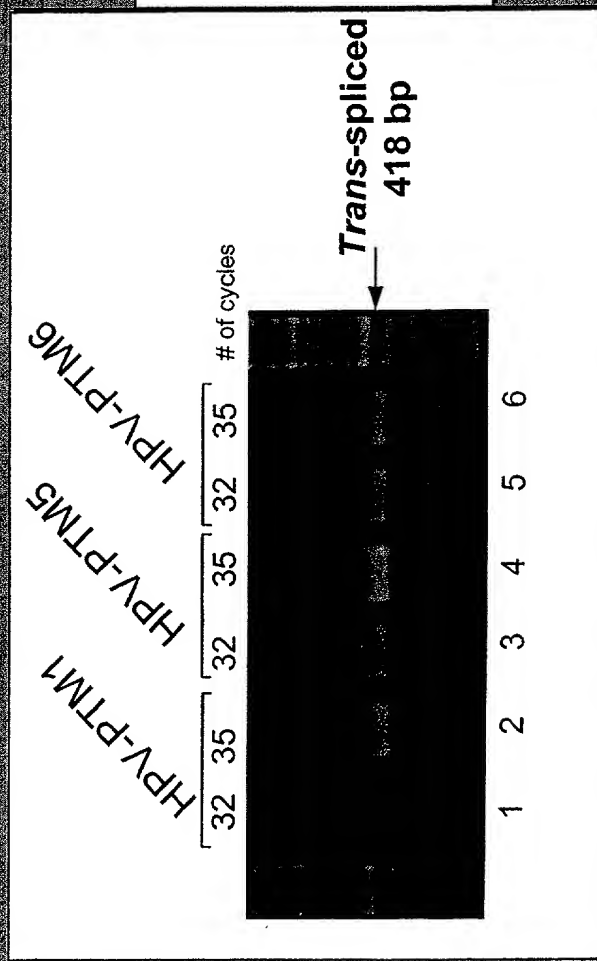
<u>PTM</u>	<u>% trans-spliced</u>
pcDNA3.1	0
HPV-PTM1	0.16
HPV-PTM5	0.12
HPV-PTM6	0.11
CF-PTM27	0

Quantification of *trans*-splicing efficiency using real-time QRT-PCR

FIG.61

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Trans-splicing Efficiency of HPV-PTM1, 5, & 6 in SiHa Cells



• SiHa cells transfected with 1.5 µg plasmid DNA, LipoPlus
• RNA isolated after 48 hr

• Total RNA: 500 ng/Rxn
• Primers: oJMD15 + Lac16R
• Expected product: 418 bp

1, 3, 5, : 32 cycles
2, 4, 6, : 35

INTRON

FIG. 62

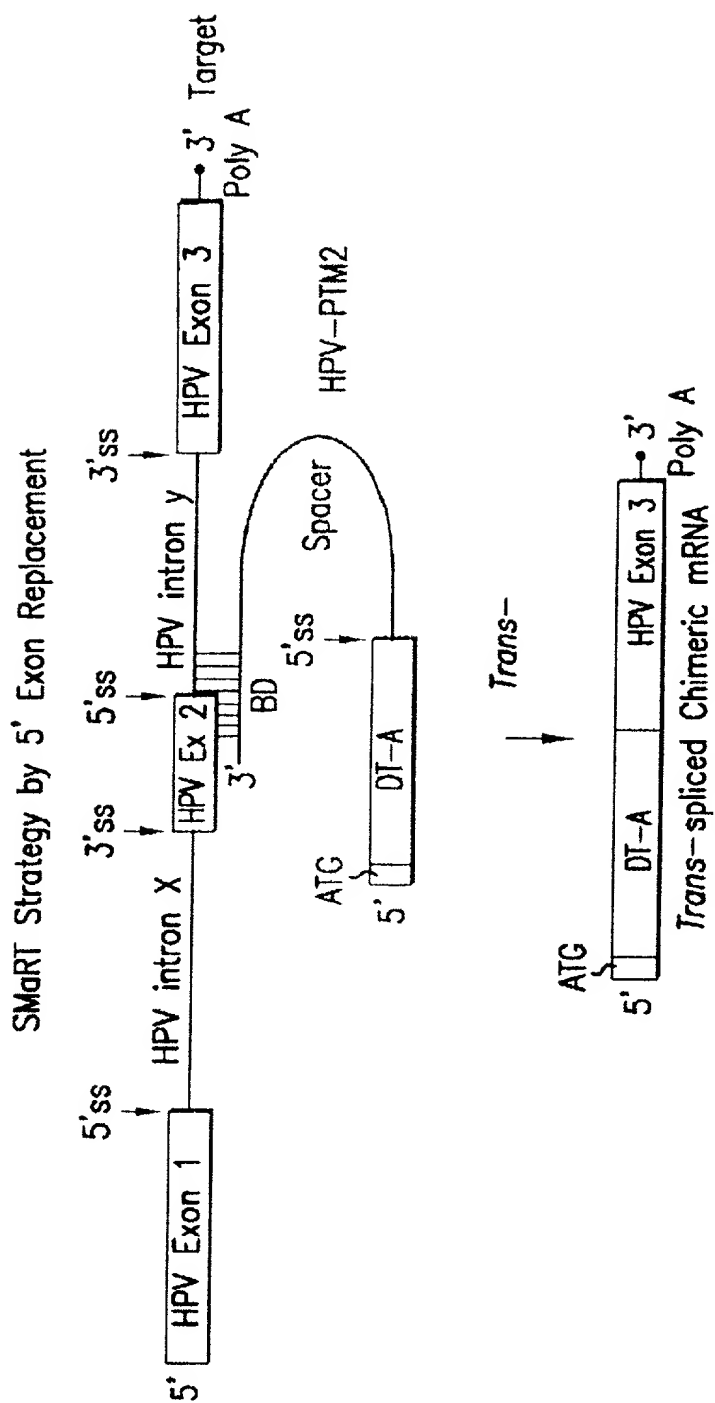
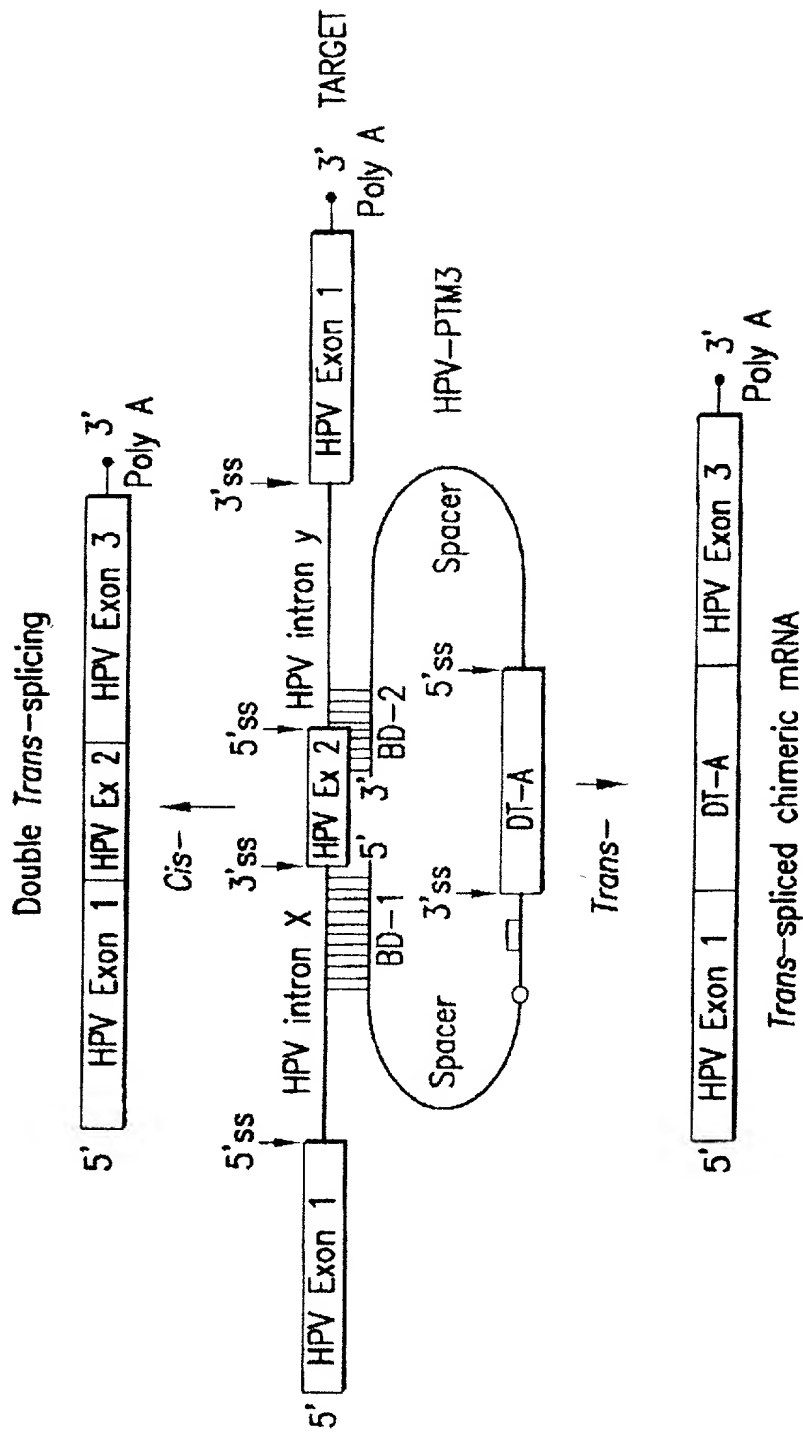


FIG. 64



Schematic diagram of a double *Trans*-splicing PTM binding to the 3' and 5' splice sites of the HPV mini-gene target

FIG.65

SMaRT Strategy by 3' Exon Replacement: Schematic diagram of a PTM binding to the 3' splice site of the HPV mini-gene target

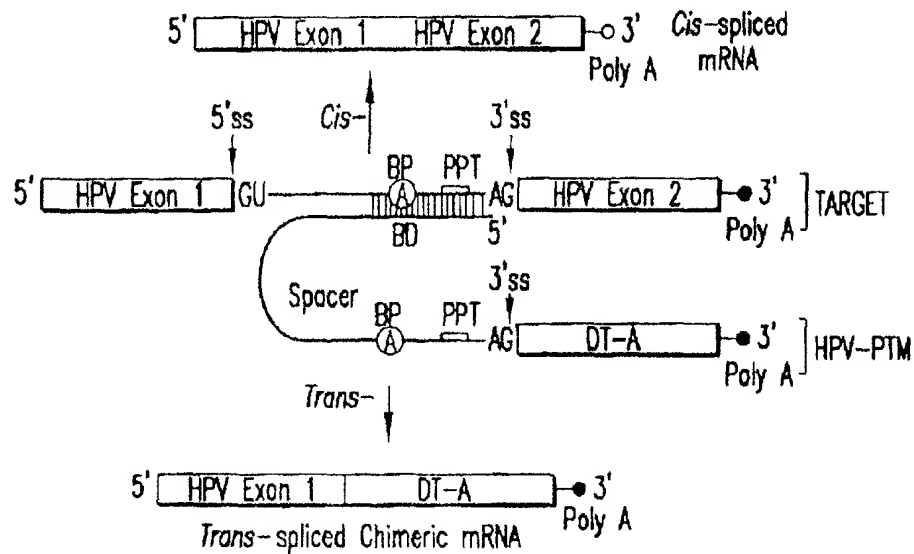


FIG.66A

SMaRT Strategy by 5' Exon Replacement: Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

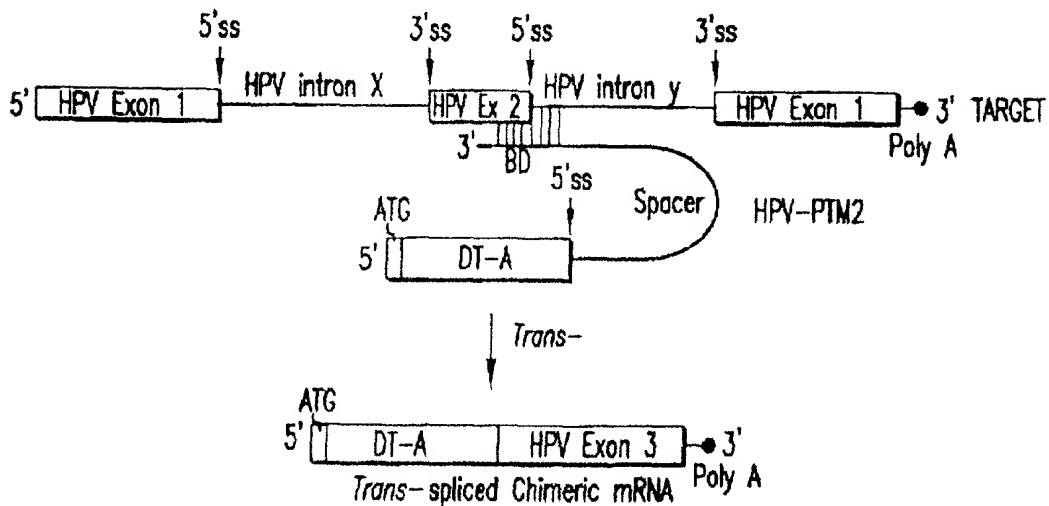


FIG.66B

HPV-PTM3 (For internal exon replacement)

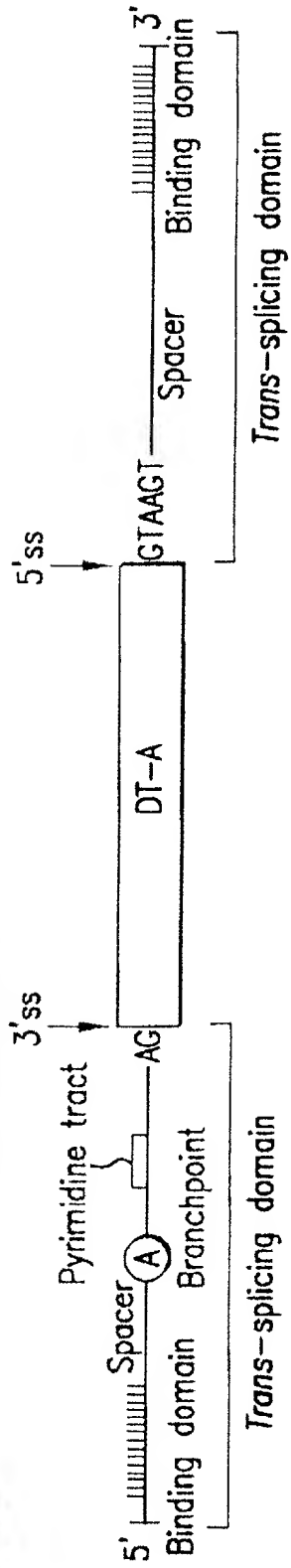


FIG.67

91 8 91